VOLUME VIII OCTOBER, 1937 No. 3

637.127.6

163. A NEW METHOD FOR THE DETERMINATION OF BUTTERFAT IN DAIRY PRODUCTS

BY JOHN GOLDING
National Institute for Research in Dairying, University of Reading

(With 2 Figures)

INTRODUCTION

As a member of Committees of the Empire Marketing Board and subsequently of the British Standards Institution, the author has had experience in methods of milk testing which has led to the discovery of a simplified test for butterfat, which may be of value in filling some of the gaps in the application of the present known methods.

To those who wish to produce "graded cream", "tea cream" and the like, the rapidity of single determinations, accuracy of results, and ease of manipulation will recommend the new method, especially as the small capital outlay makes it cheaper than even the Gerber or Babcock methods.

The need for a new method, such as that about to be described, is perhaps greatest in the standardization of ice-cream mixes, where the standard acid methods may give unsatisfactory results, and where the Mojonnier apparatus, designed to overcome the difficulty, is beyond the reach of most dairy managers in this country.

Origin of the new method

The first step in the development of the new method was the gravimetric, in place of the volumetric, estimation of the solidified fat obtained in the Alfa-Laval method(1). In the Alfa-Laval method the milk solids are dissolved in sulphuric acid and the fat separated at 70° C. (158° F.), in a separator running at 4500 rev. for 5 min.; the hot water running through the special bowl of the separator is then replaced by water at 12° C. (54° F.) and the fat solidified.

It was found possible to remove the rubber stopper of the small butyrometers, wash away the acid liquid, and leave the fat in position in the graduated neck of the butyrometer tubes.

When the tubes were air dried, satisfactory results were obtained, and as only 1·59 g. of milk is used this provides a rapid semi-micro-gravimetric method for the estimation of fat in milk in which the necessity for graduated butyrometers is avoided. Slightly low results are, however, obtained when the fat is completely dried, due partly to the loss of volatile fatty acids produced by the action of the sulphuric acid on the fat(2). Owing to the restrictions imposed by the use of these small butyrometers larger butyrometers fitted

with removable caps were next designed and a special bowl was made to hold them. With these bottles alkaline solutions such as those recommended for the Hoyberg and Hauptner tests could now be used.

By drying the fat before weighing the source of error introduced by the inflation of the fatty layer in the "Neusal" (3), Hoyberg (4) and other alkaline tests for fat in milk disappears with the volatilization of the alcohol dissolved in the fat.

The next step in the development of the new method was to follow Hoyberg's example in leaving the separation of the alcohol-inflated fat layer to gravity alone, thus dispensing with the centrifuge altogether. At this stage the treatment with hot alkaline solutions was conducted in Erlenmeyer conical flasks. After dissolving the milk solids in hot alkaline solutions the flasks were covered with loosely fitting caps which had been drawn out to a fine tube at the upper end. The flasks were then placed in a hot-water bath and weighted with a lead ring, to keep them down. Hot water was then run in till the fat, floating on the top, occupied a position about $\frac{1}{4}$ in. from the top of the cap, at which level the water-bath overflowed. After all the fat had risen, it was set solid by running ice-cold water into the water-bath.

The cap containing the solidified fat was then removed, melted out into a flat-bottomed dish and the cap, dish and fat dried to constant weight. This technique gave encouraging results, but the method was somewhat tedious.

While using the reagents "A" and "B" recommended by Overman & Garrett (5) for this technique it was found that the reagent "A" alone, if used in adequate quantities, completely dissolved the casein at room temperatures, and, what was more remarkable, the churnability was so increased that 100% of the fat could be separated in a few minutes by shaking cream with this solution followed by the addition of "breaking" water.

As all the butterfat came out in a lump the use of hot water and a cap was soon found to be unnecessary and the method was thus further simplified. The butter rises at once to the surface, and as all the fat globules are aggregated, the period required in the Hoyberg test for small fat globules to rise was found to be no longer necessary. If the bottle or flask in which the churning is carried out is filled up with cold water, the butter can easily be removed to a metal dish for drying off the water and alcohol before cooling and weighing. The previous treatment with solution A also freed the butter fat from other impurities.

Reagent for new test

The reagent "A" was originally recommended by Overman & Garrett (5) for use with a reagent "B" for "a non-acid Babcock method for determining fat in ice cream".

The test was volumetric and was conducted in Babcock bottles heated to boiling-point in a water-bath, spun in a centrifuge, the fat then being read off by volume.

The composition of reagent "A" was given as follows:

75 ml. of C.P. ammonium hydroxide.
35 ml. of *n*-butyl alcohol.
15 ml. of 95% ethyl alcohol or denatured grain alcohol.

In the new test it was found that commercial reagents gave as good results as analytical reagents. The cost was as follows:

1 Winchester quart ammonia 0·880 2*s.* 9*d.*
1 Winchester quart *n*-butyl alcohol 10*s.*
1 gall. industrial alcohol 4*s.* 6*d.*

Made up in the above proportions a litre of solution "A" cost 2*s.*, this being enough for 100 tests on cream using 10 g. of cream per test, or about ¼*d.* per test for reagents. If 10 ml. of a dilution of 50 parts solution "A" in 50 parts of water is used as "break" water a further ⅛*d.* is added to the cost of the test. As the tests gave entirely satisfactory results and the cost was so low it was not thought necessary to explore the use of other possible reagents at present.

APPARATUS

The glassware

No special or graduated glassware is necessary for the new test.

Cream, ice-cream mix, and milk can readily and quickly be churned by shaking the mixture violently in a wide-mouthed, corked bottle for a few minutes by hand. For dairy work, a well-corked half-pint milk bottle will be found a most convenient container. The quantity taken may be varied to suit local requirements; 10 g. or multiples of 10 g. of cream or milk will simplify calculations.

The balance

The scale of the operation is determined by the accuracy of the balance available. A balance and weights giving accurate readings to 1 cg. or better will, however, be required. In laboratories where air-damped balances weighing to 4 places of decimals of a gram are available good results (see table) can be obtained on 2 g. of cream or upwards. For this 2 or 3 oz. bottles are convenient. With these quantities the fat may be dried in aluminium bottle caps, as has been recommended (6) for total solids in 1 g. of milk, where air-damped balances are available. Such quantities will be found most convenient, economical and accurate.

Where less accurate balances only are available, quantities of milk or cream yielding 1–5 g. of butterfat must be taken. It is then necessary to use metal (nickel or aluminium), flat-bottomed dishes 3 in. in diameter and 1 in. deep. To remove the fat from the bottle to the dish, moistened wooden spatulas have been found most convenient. For small-scale work moistened orange sticks will serve, but for larger quantities wooden spoons or spatulas made from old "scotch hands" are admirable as the butter does not stick to the moistened wood.

The dish of butter must be dried on a hot plate (about 135° C.) and/or in an oven, to constant weight. Methods for determining water in butter are applicable. A thick piece of metal heated with a low flame will serve if spitting of the fat is avoided. The alcohol dissolved in the fat aids the drying, and freedom from the smell of butyl alcohol indicates the end of drying.

Additional apparatus

For convenience in taking the 10 g. of material for the test an 11 ml. milk syringe fitted with a screwed piston rod and setting and locking nuts on the outside of the syringe is of great help in this work (Fig. 1). The nuts are adjusted so that the full stroke of the syringe delivers 10 g. of milk or of cream. The weight to the nearest centigram is finally adjusted on the balance if different products are being tested; but with milk the weighing of the sample is unnecessary if the syringe has been carefully set.

Fig. 1.

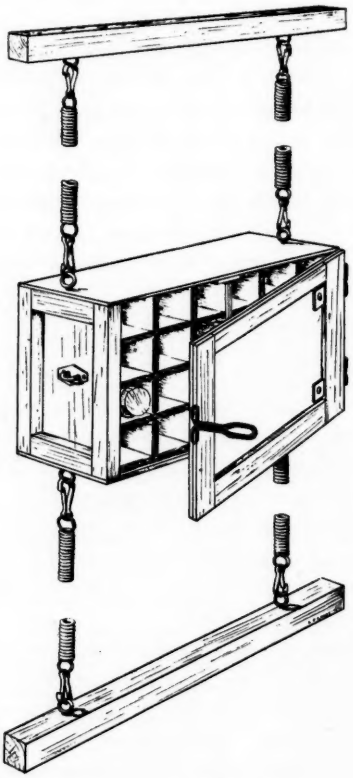


Fig. 2.

The final cubic centimetre contains any bubbles which may have to be drawn in with the milk. This volume must be ejected before taking a fresh sample and the syringe rinsed out with two or three strokes while immersed in the fresh sample. Before taking the final measure the syringe is inverted with the nozzle placed into the opening of an unspillable ink bottle or similar container held upside down. The air and froth are ejected into the bottle

which serves to catch it. Such a syringe makes it possible to take samples of milk into the churning bottles, from the milk of individual cows in the cow byre, or from bulk milk on the receiving platform where only single determinations of fat are required. The weight delivered is so constant that weighing may be omitted.

THE SPRING CHURN

In our experiments a simple but very efficient churn was improvised for churning twenty-four 2 oz. sample bottles of cream or milk at one time. This churn (Fig. 2) was made[1] by attaching a partitioned sample box to two springs above and two springs below stretched between an upper and a lower beam. The churn was operated by giving the box an occasional downward push with the hand to keep the oscillations uniform, at about 100 times up and down per minute. In this churn the bottles were generally churned in a horizontal position.

THE METHOD

The method is very adaptable. The criteria for successful results are (1) a clear or slightly opalescent serum when the test is completed, (2) a firm lump of butter or large granular lumps which can easily be removed.

The following procedure is recommended for the determination of fat in cream or ice-cream mix where a balance weighing accurately to 1 cg. or better is available:

(1) Weigh an empty $\frac{1}{2}$ pt. milk bottle to the nearest centigram.

(2) Add 10 g. of 25% cream or of 8–12% ice-cream mix, and weigh or adjust.

If the same product is being constantly tested the syringe mentioned above, set to deliver 10 g., may be used with or even without weighing.

(3) Add an approximately equal volume of solution "A", fix a wetted cork firmly in the neck of the bottle, shake for about 10 sec. till well mixed.

(4) Add about 10 ml. of a mixture of equal parts solution "A" and water cleared with a few drops of alcohol. Use this solution to wash the cork and inside of the bottle into the liquid.

(5) Churn by shaking violently till the butter separates (about 1–2 min.). This may be done in batches in a spring churn or other suitable rapid churn.

(6) Add about 50 ml. of water, again washing the cork and sides of the bottle.

(7) Churn by hand with a circular movement, which forms the butter into a lump.

(8) Fill up with water, using ice-cold water if the butter is soft, as may happen in very hot rooms. The solid butter should float above the rim of the neck of the bottle.

[1] Mr Wagstaff procured the springs at 6*d*. each from Woolworth's and made the churn.

(9) Carefully remove every particle of butter to the metal dish, using the wetted wooden spatula described above.

(10) Dry on a hot plate or water-bath till free from smell of butyl alcohol. Drying may be finished in the steam or electric oven.

(11) Cool; if the dish is placed on a thick metal slab it will cool quickly.

(12) Weigh, subtract weight of dish or use a tare, and calculate the percentage of fat. The fat should not lose weight after further heating for ½ hr., but weighing till constant weight can be avoided when the method has been tested. With one weighing the test can be done in less than half an hour.

Table I gives a number of satisfactory results obtained in working out the new method. The table indicates that the method is applicable to creams, ice-cream mixes, and milks varying in fat content.

Table I. *Satisfactory results obtained in working out the new method using variations in technique described in the text*

				New method			
Sample no.	Material	Average Röse Gottlieb	Average Gerber	Average % fat	Agreement	No. of tests	Weight of material g.
1	Thick cream	58·28	58·25	58·16	±0·35	9	5
2	,,	—	55·14	55·10	±0·38	6	2–11
3	Cream	39·33	39·25	39·11	±0·09	6	1·8–10·1
4	,,	25·72	25·6	25·67	±0·12	8	2
5	,,	25·72	25·6	25·51	±0·15	4	20
6	,,	25·31	25·12	25·26	±0·05	3	5
7	,,	24·58	25·0	24·56	±0·07	3	10, 30 and 40
8	Thin cream	12·73	12·5	12·45	±0·49	19	10–40
9	,,	—	13·4	13·33	±0·06	5	10
10	,,	8·17	8·0	8·02	±0·05	4	4
11	Vanilla ice-cream mix	8·27	7·6	8·27	±0·08	6	5·5
12	,,	8·16	7·9	8·2	±0·05	8	2·18
13	Strawberry ice-cream mix	10·32	—	10·26	±0·12	6	5·43
14	Vanilla ice-cream mix	10·80	11·0	10·86	±0·05	8	10
15	Chocolate ice-cream mix	12·95	11·7	12·98	±0·21	6	10
16	Milk	4·59	4·66	4·57	±0·01	24	10
17	,,	4·38	4·42	4·36	±0·04	19	10
18	,,	4·24	4·25	4·22	±0·07	24	10
19	,,	2·67	2·62	2·60	±0·03	24	10
20	,,	—	1·15	1·15	±0·08	11	10

Preliminary experiments have indicated that it is also applicable to other dairy products. With cream cheese and even with hard cheese it has been found possible to obtain promising results.

With different products the technique given above requires some slight modification; for instance, in testing milk the best results were obtained by adding to the weight of milk used about half the volume of solution "A", instead of an equal volume as in the case of cream and ice-cream mix.

Larger amounts than are necessary to obtain a clear serum at the end of the operation should be avoided. The average Röse Gottlieb results given in Table I, column 3, are taken to represent the true percentage of fat in the sample. Most of the averages are of triplicate determinations agreeing closely between themselves. The average Gerber agrees fairly closely in most cases.

With rich milks (upwards of 5% fat) the Gerber results are always higher (about 0·1%) than the Röse Gottlieb, and with poor milks the Gerber figure is lower than the Röse Gottlieb. The limit of error is not more than ±0·05. Good agreement is not obtained between Röse Gottlieb and Gerber results on ice-cream mix, as is seen in Table I. The average results, obtained by the new method, Table I, column 5, are calculated from the number of tests given in column 7; the agreement between these tests is shown in column 6. This figure is half the difference between the highest and lowest figures obtained; the majority agreed very closely with the average.

The weight of material taken, shown in the last column, varied considerably, but within these limits the weight taken did not affect the result if the volume of solution "A" was used in proportion.

The experiments indicated that it is well not to use less of solution "A" than half the volume of milk taken, as with lower quantities a clear serum is difficult to obtain. In other experiments with cream cheese more was required and with hard cheese it was necessary to use up to five times the volume of the cheese taken (2 g. cheese to 10 ml. of solution "A"). A "breaking" liquid made of equal parts of solution "A" and water cleared with a few drops of industrial alcohol, was better than water alone in giving a clear serum. A volume equal to that of the material taken is as a rule sufficient.

Dilution of cream or ice-cream mix before adding solution "A" is not advised; some good results were, however, obtained when less water than the volume of the cream was used.

Much more experimental work suggests itself, but sufficient has been done to inspire confidence in introducing the method at this stage. It is hoped that those who make use of it will check their results against standard Röse Gottlieb method from time to time.

The author wishes to thank Mr Alfred Wagstaff, by whose loyal assistance most of the practical work has been carried out.

REFERENCES

(1) GOLDING (1934). *Agric. Progr.* **11**, 116–19.
(2) BAILEY (1919). *J. Dairy Sci.* **2**, 331–73.
(3) GOLDING (1911). *Analyst*, **36**, 203–6.
(4) GOLDING (1928). *J. Brit. Dairy Fmrs' Ass.* **40**, 69–72.
(5) OVERMAN & GARRETT (1930). *Bull. Ill. agric. Exp. Sta.* No. 360.
(6) GOLDING (1934). *Analyst*, **59**, 468–74.

(*MS. received for publication* 20 *September* 1937)

612.392.84

164. THE NUTRITIVE VALUE OF MILK SUPPLEMENTED WITH MINERALS AS AN EXCLUSIVE DIET FOR RATS. COMPARISON OF EQUAL VOLUMES OF SUMMER AND "WINTER" MILK BEFORE AND AFTER LABORATORY PASTEURIZATION[1]

By K. M. HENRY, E. W. IKIN and S. K. KON
From the National Institute for Research in Dairying, University of Reading

(With 2 Figures)

In comparing winter and summer milks Elvehjem *et al.* (2) reported differences in favour of the latter in the rate of growth of rats given unlimited access to an exclusive diet of milk supplemented with minerals.

These authors also noticed differences in growth in favour of raw milk when milk produced under stall-feeding conditions was given to rats, before and after laboratory pasteurization.

We have recorded in a previous publication (Henry & Kon (3)) our failure to confirm the latter findings when we fed to rats equal volumes of the raw and pasteurized milks, though the milk was produced under conditions of stall-feeding.

The experiments of Elvehjem *et al.* (2) on the seasonal variations in the nutritive value of milk were carried out at different times of the year and therefore involved indirect comparisons of rates of growth. They may have been complicated by seasonal differences in the rats themselves.

For this reason we availed ourselves of the opportunity which arose in connexion with other work (Campion *et al.* (4)) to compare simultaneously by our technique milks produced under summer and under winter conditions.

The milks, which were produced in May, June and July, were obtained from two groups, each consisting of two Shorthorn cows. One group was at pasture with the herd, the cows of the other group had received normal winter rations since the previous autumn and had been kept indoors since the end of February and the end of March respectively. The details of feeding and management of the cows are given in our previous paper (Campion *et al.* (4)).

Experimental

(1) *Source and preparation of the milks*

Milks from the cows of groups 1 and 3 of the above paper were used.

The evening and morning milkings from each group were collected separately and the fat percentage was determined. In each group the morning and

[1] Read, in part, before the Biochemical Society, 17 October 1936 (Henry & Kon (1)).

evening milks were then blended so that the blended milks had the same fat content.

The fat contents naturally varied from day to day. In general there was very close agreement between the calculated fat percentage of the two blended milks and the actual figure obtained. If, as occasionally happened, the two milks differed in fat percentage this was allowed for on the next day, so that the average fat figures for the whole period were practically identical. As it was sometimes impossible to blend to the same figure, allowance was made for this at the earliest possible date. Table I gives the daily figures for the percentage of fat (estimated and obtained) and total solids of the two milks.

Table I. *The percentages of fat and total solids in the experimental milks*

	Summer milk			"Winter" milk		
	% fat		% total	% fat		% total
Date	Estimated	Found	solids	Estimated	Found	solids
4. v. 36	3·00	3·10	—	3·00	3·05	—
5. v. 36	3·40	3·45	12·49	3·40	3·45	12·10
6. v. 36	3·20	3·25	12·19	3·20	3·25	11·83
7. v. 36	3·30	3·35	12·38	3·30	3·35	12·11
8. v. 36	3·30	3·35	12·32	3·30	3·25	11·98
9. v. 36	3·50	3·65	12·52	3·50	3·55	12·10
10. v. 36	3·50	3·65	12·52	3·50	3·55	12·10
11. v. 36	3·80	3·85	—	3·80	3·75	—
12. v. 36	3·55	3·60	12·36	3·45	3·45	12·09
13. v. 36	3·55	3·55	12·34	3·65	3·75	12·53
14. v. 36	3·20	3·25	12·10	3·25	3·25	11·95
15. v. 36	3·30	3·35	12·24	3·40	3·45	12·03
16. v. 36	3·50	3·55	12·24	3·60	3·65	12·02
17. v. 36	3·50	3·55	12·24	3·60	3·65	12·02
18. v. 36	3·20	3·25	12·15	3·20	3·20	11·72
19. v. 36	3·25	3·20	12·27	3·15	3·15	11·84
20. v. 36	3·50	3·60	12·52	3·60	3·65	12·33
21. v. 36	3·65	3·65	12·53	3·60	3·65	12·42
22. v. 36	3·55	3·55	12·38	3·55	3·60	12·30
23. v. 36	3·50	3·55	12·46	3·50	3·55	12·23
24. v. 36	3·50	3·55	12·46	3·50	3·55	12·23
25. v. 36	3·30	3·35	12·21	3·30	3·30	11·95
26. v. 36	3·60	3·60	12·40	3·60	3·40	12·05
27. v. 36	3·40	3·50	12·22	3·50	3·60	12·25
28. v. 36	3·60	3·60	12·44	3·70	3·65	12·35
29. v. 36	3·30	3·35	12·22	3·30	3·40	12·03
30. v. 36	3·30	3·35	12·22	3·30	3·40	12·03
31. v. 36	3·30	3·30	12·11	3·30	3·30	12·93
1. vi. 36	3·30	3·30	12·11	3·30	3·30	12·93
2. vi. 36	3·40	3·40	12·21	3·40	3·30	12·00
3. vi. 36	3·40	3·45	12·16	3·50	3·50	12·20
4. vi. 36	3·50	3·50	12·30	3·50	3·40	12·05
5. vi. 36	3·45	3·45	12·28	3·45	3·45	11·96
6. vi. 36	3·60	3·70	12·36	3·70	3·70	12·34
7. vi. 36	3·60	3·70	12·36	3·70	3·70	12·34
8. vi. 36	3·40	3·55	12·37	3·60	3·65	12·37
9. vi. 36	3·65	3·65	12·43	3·70	3·70	12·46
10. vi. 36	3·40	3·40	12·21	3·45	3·45	12·21
11. vi. 36	3·40	3·55	12·34	3·60	3·65	12·47
12. vi. 36	3·50	3·50	12·34	3·25	3·25	12·09
13. vi. 36	3·20	3·20	12·04	3·30	3·30	11·99
14. vi. 36	3·20	3·20	12·04	3·30	3·30	11·99
15. vi. 36	3·70	3·65	12·40	3·70	3·75	12·38
16. vi. 36	3·35	3·35	12·15	3·40	3·45	11·18
17. vi. 36	3·60	3·55	12·29	3·40	3·45	12·07
18. vi. 36	3·50	3·55	12·22	3·30	3·25	11·91

Table I (*continued*)

Date	Summer milk % fat Estimated	Summer milk % fat Found	Summer milk % total solids	"Winter" milk % fat Estimated	"Winter" milk % fat Found	"Winter" milk % total solids
19. vi. 36	3·60	3·60	12·11	3·55	3·55	12·25
20. vi. 36	3·40	3·40	11·89	3·50	3·45	12·01
21. vi. 36	3·40	3·40	11·89	3·50	3·45	12·01
22. vi. 36	3·50	3·55	12·04	3·60	3·65	12·10
23. vi. 36	3·75	3·75	12·37	3·60	3·60	12·23
24. vi. 36	3·50	3·45	12·05	3·70	3·70	12·07
25. vi. 36	3·70	3·70	12·51	3·60	3·60	12·17
26. vi. 36	3·60	3·55	12·30	3·60	3·50	12·07
27. vi. 36	3·70	3·70	12·54	3·70	3·70	12·24
28. vi. 36	3·70	3·70	12·54	3·70	3·70	12·24
29. vi. 36	3·80	3·75	12·51	3·80	3·75	12·38
30. vi. 36	3·80	3·75	12·51	3·80	3·75	12·38
1. vii. 36	3·70	3·80	12·45	3·70	3·70	12·29
2. vii. 36	3·70	3·80	12·45	3·70	3·70	12·29
3. vii. 36	3·70	3·70	12·23	3·80	3·80	12·40
4. vii. 36	3·70	3·70	12·23	3·80	3·80	12·40
5. vii. 36	3·70	3·70	12·23	3·80	3·80	12·40
6. vii. 36	3·55	3·55	12·17	3·25	3·25	11·90
7. vii. 36	3·55	3·55	12·17	3·25	3·25	11·90
8. vii. 36	3·00	3·05	11·74	3·30	3·30	11·92
9. vii. 36	3·00	3·05	11·74	3·30	3·30	11·92
10. vii. 36	3·35	3·35	12·08	3·40	3·40	12·12
11. vii. 36	3·35	3·35	12·08	3·40	3·40	12·12
12. vii. 36	3·35	3·35	12·08	3·40	3·40	12·12
13. vii. 36	3·80	3·85	12·44	3·80	3·85	12·45
14. vii. 36	3·80	3·85	12·44	3·80	3·85	12·45
Average	3·48	3·51	12·27	3·50	3·51	12·13

After blending, half of each milk was pasteurized in the laboratory. The procedure was as follows: The milks were placed in aluminium saucepans with lids through which was passed a rod attached to a perforated aluminium stirrer. The saucepans were then placed in gently boiling water and the temperature raised to about 148° F. (never less than 145 or more than 150) in the course of 8–10 min., the milk being gently stirred all the time. The pans were then quickly transferred to a "holder tank" in which the temperature of the water had been previously raised to 150° F. The milk was "held" for 30 min., during which time it was occasionally stirred. During the period of "holding" the temperature of the pasteurizer fell a few degrees, but never below 145° F. The milks were then cooled with continuous stirring as rapidly as possible in running water, to which some ice had been added, to approximately their initial temperature. Throughout pasteurization care was taken not to break the surface of the milk while stirring. To check the efficiency of pasteurization phosphatase tests (Kay & Graham (5)) were carried out twice weekly and were negative throughout the experiment. On Saturdays sufficient milk for use on Saturday and Sunday was pasteurized, and both raw and pasteurized milks for use on Sunday were kept in cold storage overnight.

(2) *The feeding of the milks*

(*a*) *Exclusive diet of milk supplemented with minerals.*

Twelve groups of four litter-mate male rats were placed, when 22–24 days old and weighing 45–58 g., in individual cages on screens. The rats were assigned by drawing lots to either (i) raw summer milk, (ii) pasteurized summer milk, (iii) raw "winter" milk, or (iv) pasteurized "winter" milk. The milk intake was equalized within fours, the rat with the poorest appetite setting the pace. The milk was fed in stainless steel containers. The salts were given as a solution 1 ml. of which was first offered mixed with 5 ml. of the appropriate milk, and when this had been consumed the balance of the day's ration was offered. Any milk unconsumed by the following morning was measured back and a corresponding additional amount offered with the next ration of milk. This ration was cut down for the group if the amount left by any rat exceeded 5 ml. If all rats in the group cleared up their allowance the next day's ration was increased. The salt mixtures were as used by Elvehjem *et al.* (2) and by us (3). The trays under each cage were cleaned out twice a week and a record of the rats which scoured was kept. At the end of 8 weeks the rats were killed by coal gas and the length from the tip of the nose to the centre of the anus was measured according to Donaldson (6). The intestinal tract was carefully freed from all adhering fat and discarded. The carcasses were then analysed for fat and moisture as already described by Henry & Kon (3). This experiment lasted from 4 May to 30 June 1936.

The results are shown in Tables II, II*a* and III. Table II gives the initial and final weights, the total and daily gain for the whole period and for the final 6 weeks, the length, the weight : length ratio, the milk intakes and refusals and the gain in weight per gram solids ingested. Table II*a* gives the total amounts of milk refused by each rat. Table III gives the analyses of the carcasses for fat and moisture. The results of the statistical tests are given in Tables IV, IV*a* and IV*b*. The growth curves are in Fig. 1. A description of the statistical methods is given partly in a previous publication (White (7)) and partly in a paper by White (16) in this issue of the *Journal*.

It will be seen from Fig. 1 that the growth curves of the four groups of rats lie very close together, while Table II shows that the gain over the 8-week period is similar for all groups, a slight superiority being shown by the two groups on pasteurized milk. During the course of the experiment two rats (No. 5387, on pasteurized "winter" milk and No. 5430, on pasteurized summer milk) consistently left large quantities of milk and were finally left out of their groups, as it was considered that their litter-mates were not being given a fair chance of growth. The two groups, each now reduced to three rats, were continued as before, and the two rats left out were allowed milk *ad lib.* Averages were made which included these two groups of rats in the one case and excluded either one or both groups in the others (Tables II and III).

Table II. *Weights, gains in weight, daily gains in weight, body lengths, weight : length ratios, milk intakes, gains per gram solids ingested, and milk refusals of the experimental rats on exclusive milk diets*

Litter no.	Rat no.	Weight (g.) Initial	Weight (g.) 2 weeks	Weight (g.) 8 weeks	Gain (g.) 8 weeks	Gain (g.) Final 6 weeks	Gain per day (g.) 8 weeks	Gain per day (g.) Final 6 weeks	Length when killed (mm.)	Wt./length ratio	Milk intake (ml.)	*Gain per gram solids ingested (g.)	†Milk refusals
						Summer raw							
I	5332	50	71	181	131	110	2·34	2·62	194	0·93	3606·5	0·2870	28
II*a*	5338	53	73	155	102	82	1·82	1·95	185	0·84	3600·0	0·2238	23
II*b*	5341	58	67	142	84	75	1·50	1·79	182	0·78	3380·0	0·1963	6
III	5348	46	49	162	116	113	2·07	2·69	194	0·84	3870·0	0·2368	9
IV	5386	52	60	159	107	99	1·91	2·36	196	0·81	3587·5	0·2356	2
V	5393	55	78	173	118	95	2·11	2·26	194	0·89	3720·0	0·2506	6
VI	5419	50	64	160	110	96	1·96	2·29	191	0·84	3665·0	0·2371	4
VII	5429	54	71	194	140	123	2·50	2·93	192	1·01	4240·0	0·2609	0
VIII	5398	49	71	158	109	87	1·95	2·07	194	0·81	3820·0	0·2254	35
IX	5405	46	75	190	144	115	2·57	2·74	198	0·96	4395·0	0·2588	6
X	5426	52	61	182	130	121	2·32	2·88	199	0·91	4085·0	0·2514	9
XI	5453	55	66	158	103	92	1·84	2·19	185	0·85	3610·0	0·2254	2
Average I		51·7	67·2	167·8	116·2	100·6	2·07	2·40	191·8	0·873	3798·3	0·2408	130
,, II		51·6	67·8	168·6	117·0	100·7	2·09	2·40	191·5	0·878	3817·4	0·2412	128
,, III		51·5	66·8	165·5	114·0	98·6	2·04	2·35	191·8	0·860	3758·1	0·2389	130
,, IV		51·4	67·5	166·1	114·7	98·5	2·05	2·35	191·4	0·865	3775·2	0·2393	128
						Summer pasteurized							
I	5334	53	72	168	115	96	2·05	2·29	193	0·87	3620·0	0·2510	18
II*a*	5337	57	80	174	117	94	2·09	2·24	194	0·90	3600·0	0·2568	3
II*b*	5342	57	70	149	92	79	1·64	1·88	185	0·81	3380·0	0·2150	29
III	5347	45	50	162	117	112	2·09	2·67	190	0·85	3870·0	0·2388	16
IV	5384	54	70	154	100	84	1·79	2·00	189	0·81	3582·5	0·2205	3
V	5391	56	77	172	116	95	2·07	2·26	194	0·89	3720·0	0·2463	25
VI	5418	52	70	166	114	96	2·04	2·29	191	0·87	3655·0	0·2464	3
VII	5430‡	49	68	182	133	114	2·38	2·71	190	0·96	4057·5	0·2590	28
VIII	5402	51	70	178	127	108	2·27	2·57	196	0·91	3830·0	0·2620	5
IX	5408	46	82	200	154	118	2·75	2·81	204	0·98	4395·0	0·2768	3
X	5421	52	65	182	130	117	2·32	2·79	199	0·91	4090·0	0·2511	5
XI	5457	52	62	151	99	89	1·77	2·12	188	0·80	3610·0	0·2167	2
Average I		52·0	69·7	169·8	117·8	100·2	2·11	2·39	192·8	0·897	3784·2	0·2450	140
,, II		51·8	69·6	171·3	119·5	101·6	2·13	2·42	193·1	0·886	3802·5	0·2473	137
,, III		52·3	69·8	168·7	116·5	98·9	2·08	2·36	193·0	0·873	3759·3	0·2438	112
,, IV		52·1	69·8	170·2	118·1	100·4	2·11	2·39	193·4	0·879	3777·0	0·2461	109
						"Winter" raw							
I	5333	54	66	173	119	107	2·13	2·55	196	0·88	3620·0	0·2634	0
II*a*	5336	56	76	167	111	91	1·98	2·17	193	0·87	3600·0	0·2471	11
II*b*	5340	57	74	145	88	71	1·57	1·69	184	0·79	3380·0	0·2087	3
III	5344	48	56	164	116	108	2·07	2·57	191	0·86	3870·0	0·2403	20
IV	5385	55	70	155	100	85	1·79	2·02	184	0·84	3585·0	0·2236	26
V	5390	58	86	174	116	88	2·07	2·10	193	0·90	3718·0	0·2501	27
VI	5415	49	73	166	117	93	2·09	2·21	185	0·90	3660·0	0·2562	21
VII	5433	52	71	202	150	131	2·68	3·12	201	1·00	4239·5	0·2836	24
VIII	5403	55	67	160	105	93	1·88	2·21	195	0·82	3830·0	0·2197	3
IX	5407	46	73	198	152	125	2·71	2·98	203	0·98	4395·0	0·2772	5
X	5425	53	69	186	133	117	2·38	2·79	200	0·93	4090·0	0·2606	13
XI	5455	52	63	151	99	88	1·77	2·10	182	0·83	3598·0	0·2205	17
Average I		52·9	70·4	170·1	117·2	99·7	2·09	2·37	192·3	0·883	3798·9	0·2459	170
,, II		52·7	70·5	171·5	118·7	101·0	2·12	2·41	193·0	0·887	3818·2	0·2479	144
,, III		53·0	70·4	167·2	114·2	96·8	2·04	2·31	191·5	0·873	3758·7	0·2425	146
,, IV		52·8	70·4	168·4	115·6	98·0	2·07	2·33	192·2	0·876	3776·1	0·2444	120

* The solids in milk are calculated from the data of Table I on the assumption that the specific gravity of milk is 1·03.

† Group totals are given instead of averages.

‡ These rats left milk consistently and were finally excluded from their groups (see p. 285).

Table II (*continued*)

Litter no.	Rat no.	Weight (g.) Initial	2 weeks	8 weeks	Gain (g.) 8 weeks	Final 6 weeks	Gain per day (g.) 8 weeks	Final 6 weeks	Length when killed (mm.)	Wt./length ratio	Milk intake (ml.)	*Gain per gram solids ingested (g.)	†Milk refusals
						"Winter" pasteurized							
I	5331	53	65	172	119	107	2·13	2·55	192	0·90	3620·0	0·2635	3
II*a*	5335	55	74	168	113	94	2·02	2·24	188	0·89	3600·0	0·2516	15
II*b*	5339	56	72	143	87	71	1·55	1·69	174	0·82	3380·0	0·2063	19
III	5345	47	59	165	118	106	2·11	2·52	194	0·85	3870·0	0·2444	5
IV	5387‡	54	64	150	96	86	1·71	2·05	199	0·75	3410·0	0·2257	26
V	5389	56	75	178	122	103	2·18	2·45	197	0·90	3720·0	0·2629	2
VI	5416	50	68	172	122	104	2·18	2·48	195	0·88	3660·0	0·2672	25
VII	5431	51	67	192	141	125	2·52	2·98	198	0·97	4240·0	0·2666	5
VIII	5400	52	69	168	116	99	2·07	2·36	199	0·84	3830·0	0·2428	1
IX	5411	46	70	203	157	133	2·80	3·17	201	1·01	4387·5	0·2868	29
X	5422	52	49	183	131	134	2·34	3·19	197	0·93	4086·5	0·2569	19
XI	5454	55	62	150	95	88	1·70	2·10	184	0·82	3610·0	0·2109	40
Average	I	52·3	66·2	170·3	118·1	104·0	2·11	2·48	193·2	0·897	3784·5	0·2488	189
„	II	52·1	66·4	172·2	120·1	105·6	2·15	2·52	192·6	0·892	3818·6	0·2509	163
„	III	52·4	66·1	168·4	116·0	102·1	2·07	2·43	192·7	0·872	3743·1	0·2472	184
„	IV	52·2	66·3	170·2	118·0	103·7	2·11	2·47	192·1	0·884	3776·4	0·2493	158

Average I = average for 12 animals.
Average II = average for 11 animals, omitting litter No. IV.
Average III = average for 11 animals, omitting litter No. VII.
Average IV = average for 10 animals, omitting litters Nos. IV and VII.

* † ‡ See footnotes, p. 286, for explanation.

Table II*a*. *Total amounts of milk refused by the experimental rats on exclusive milk diets*

Litter no.	Rat no.	Summer raw ml.	Rat no.	Summer pasteurized ml.	Rat no.	"Winter" raw ml.	Rat no.	"Winter" pasteurized ml.
I	5332	301·5	5334	53·5	5333	0	5331	8·0
II*a*	5338	102·5	5337	21·0	5336	42·5	5335	60·0
II*b*	5341	34·0	5342	209·0	5340	6·0	5339	94·0
III	5348	30·5	5347	84·5	5344	123·0	5345	17·5
IV	5386	13·5	5384	12·0	5385	122·0	5387	402·5*
V	5393	20·5	5391	85·5	5390	133·5	5389	8·5
VI	5419	28·0	5418	21·0	5415	86·5	5416	156·0
VII	5429	0	5430	183·0†	5433	90·0	5431	30·5
VIII	5398	291·0	5402	29·5	5403	39·0	5400	15·0
IX	5404	37·5	5408	26·0	5407	22·5	5411	159·5
X	5426	77·0	5421	32·5	5425	68·5	5422	113·0
XI	5453	12·5	5457	2·5	5455	94·0	5454	171·0

* The milk intake of this rat was not equalized with that of its litter-mates during the last week of the experiment. It was offered 170 ml. less than its litter-mates.

† The milk intake of this rat was not equalized with that of its litter-mates for 12 days in the middle of the experiment, it was subsequently offered the same amounts as its mates but their ration was not cut down if it alone in the group left milk. This rat was offered 161·5 ml. of milk less than its litter-mates.

Table III. *Weight of carcass. Amount and percentage of fat, water and dry fat-free residue in the carcasses of the experimental rats on exclusive milk diets*

Litter no.	Rat no.	Carcass wt. (g.)	Fat		Water		Dry fat-free residue	
			g.	%	g.	%	g.	%
			Summer raw					
I	5332	153	20·12	13·15	97·09	63·46	35·79	23·39
II*a*	5338	135	16·08	11·91	87·42	64·76	31·50	23·33
II*b*	5341	124	15·32	12·35	79·91	64·44	28·77	23·20
III	5348	143	20·10	14·06	89·45	62·55	33·45	23·39
IV	5386	139	14·84	10·68	91·28	65·67	32·88	23·65
V	5393	147	17·38	11·82	94·72	64·44	34·90	23·74
VI	5419	139	19·59	14·09	87·10	62·66	32·31	23·24
VII	5429	170	26·87	15·81	104·88	61·69	38·25	22·50
VIII	5398	134	14·94	11·15	86·87	64·83	32·19	24·02
IX	5405	169	32·08	18·98	100·14	59·25	36·78	21·76
X	5426	159	23·88	15·02	99·96	62·87	35·16	22·11
XI	5453	142	21·40	15·07	88·52	62·34	32·08	22·59
Average	I	146·2	20·22	13·67	92·28	63·25	33·67	23·08
,,	II	146·5	20·23	13·64	92·54	63·31	33·69	23·05
,,	III	146·8	20·71	13·95	92·37	63·03	33·74	23·02
,,	IV	144·0	19·61	13·48	91·13	63·39	33·26	23·13
,,	V	147·2	20·77	13·94	92·66	63·07	33·77	22·99
,,	VI	144·5	20·09	13·76	91·12	63·16	33·29	23·08
,,	VII	144·1	19·56	13·42	91·30	63·47	33·24	23·10
,,	VIII	144·7	20·09	13·73	91·30	63·23	33·28	23·04
			Summer pasteurized					
I	5334	148	23·20	15·68	91·66	61·93	33·14	22·39
II*a*	5337	153	19·06	12·46	98·63	64·46	35·31	23·08
II*b*	5342	126	12·69	10·07	83·11	65·96	30·20	23·97
III	5347	136	17·74	13·04	86·09	63·30	32·17	23·65
IV	5384	132	21·27	16·11	81·38	61·65	29·35	22·23
V	5391	144	19·57	13·59	91·37	63·45	33·06	22·96
VI	5418	144	18·30	12·71	92·16	64·00	33·54	23·29
VII	5430	156	25·79	16·53	95·58	61·27	34·63	22·20
VIII	5402	151	19·97	13·23	94·82	62·79	36·21	23·98
IX	5408	181	31·57	17·44	109·37	60·43	40·06	22·13
X	5421	160	25·63	16·02	99·02	61·89	35·35	22·09
XI	5457	131	13·47	10·28	86·04	65·68	31·49	24·04
Average	I	146·8	20·69	13·93	92·44	63·07	33·71	23·00
,,	II	147·8	20·96	14·01	93·01	63·05	33·85	22·94
,,	III	148·2	20·64	13·73	93·44	63·20	34·11	23·07
,,	IV	146·0	20·22	13·69	92·15	63·23	33·63	23·07
,,	V	149·4	20·93	13·80	94·18	63·19	34·30	23·01
,,	VI	147·4	20·12	13·45	93·23	63·39	34·05	23·16
,,	VII	147·0	20·47	13·76	92·76	63·22	33·77	23·02
,,	VIII	148·7	20·38	13·50	94·02	63·40	34·26	23·10

Table III (*continued*)

Litter no.	Rat no.	Carcass wt. (g.)	Fat		Water		Dry fat-free residue	
			g.	%	g.	%	g.	%
			"Winter" raw					
I	5333	151	23·63	15·65	93·36	61·83	34·01	22·52
II*a*	5336	145	19·07	13·15	92·41	63·73	33·52	23·12
II*b*	5340	124	14·95	12·06	79·11	63·80	29·94	24·15
III	5344	146	19·08	13·07	92·82	63·58	34·10	23·36
IV	5385	132	18·11	13·72	84·03	63·66	29·86	22·62
V	5390	148	18·97	12·82	94·86	64·09	34·17	23·09
VI	5415	141	19·84	14·07	88·85	63·01	32·31	22·91
VII	5433	172	23·25	13·52	109·48	63·65	39·27	22·83
VIII	5403	144	19·75	13·72	90·62	62·93	33·63	23·35
IX	5407	178	25·94	14·57	111·42	62·60	40·64	22·83
X	5425	158	20·54	13·00	101·40	64·18	36·06	22·82
XI	5455	134	20·21	15·08	84·38	62·97	29·41	21·95
Average I		147·8	20·28	13·70	93·56	63·34	33·91	22·96
,,	II	147·9	20·39	13·76	93·63	63·31	33·89	22·93
,,	III	149·2	20·48	13·70	94·43	63·31	34·28	22·99
,,	IV	145·6	20·01	13·72	92·11	63·31	33·42	22·97
,,	V	149·5	20·62	13·76	94·59	63·28	34·30	22·96
,,	VI	146·9	20·20	13·72	92·92	63·27	33·78	23·01
,,	VII	145·5	20·10	13·78	92·04	63·28	33·36	22·94
,,	VIII	147·0	20·32	13·79	92·93	63·24	33·74	22·97
			"Winter" pasteurized					
I	5331	149	27·20	18·26	87·45	58·69	34·35	23·05
II*a*	5335	145	16·73	11·54	94·22	64·98	34·05	23·48
II*b*	5339	123	15·95	12·97	78·98	64·21	28·07	22·82
III	5345	147	—	—	—	—	32·79	22·31
IV	5387	131	12·76	9·74	86·43	65·98	31·81	24·28
V	5389	155	21·40	13·81	98·10	63·29	35·50	22·90
VI	5416	140	19·27	13·76	88·39	63·14	32·34	23·10
VII	5431	164	23·97	14·62	102·27	62·36	37·76	23·02
VIII	5400	147	20·21	13·75	92·48	62·91	34·31	23·34
IX	5411	179	30·58	17·08	109·05	60·92	39·37	21·99
X	5422	159	23·99	15·09	99·68	62·69	35·33	22·22
XI	5454	134	18·00	13·43	85·91	64·11	30·09	22·46
Average I		147·8	—	—	—	—	33·81	22·91
,,	II	147·8	20·91	14·00	93·00	63·03	33·91	22·97
,,	III	149·3	—	—	—	—	34·00	22·79
,,	IV	146·3	—	—	—	—	33·46	22·90
,,	V	149·5	21·73	14·43	93·65	62·73	34·12	22·84
,,	VI	147·8	—	—	—	—	33·62	22·77
,,	VII	146·2	20·61	13·94	92·07	63·09	33·52	22·96
,,	VIII	147·9	21·48	14·41	92·70	62·77	33·71	22·82

Average I =average for 12 animals.
Average II =average for 11 animals, omitting litter No. III.
Average III =average for 11 animals, omitting litter No. IV.
Average IV =average for 11 animals, omitting litter No. VII.
Average V =average for 10 animals, omitting litters Nos. III and IV.
Average VI =average for 10 animals, omitting litters Nos. IV and VII.
Average VII =average for 10 animals, omitting litters Nos. III and VII.
Average VIII =average for 9 animals, omitting litters Nos. III, IV and VII.

Table IV. *Statistical treatment of average differences in gain in weight, in body length, in gain per gram of solids ingested, in the weight : length ratio and in the percentages of fat, water and dry fat-free residue in the carcasses of the experimental rats on exclusive milk diets*

Difference tested	Summer milk. Raw *v.* pasteurized	"Winter" milk. Raw *v.* pasteurized	Raw milk. Summer *v.* "winter"	Pasteurized milk. Summer *v.* "winter"
Gain in weight (g.):				
Average A, "R" or "S"	117·0	118·7	116·2	118·1
„ B, "P" or "W"	119·2	120·1	117·2	118·0
Difference A – B	−2·5	−1·4	−1·0	+0·1
S.E.M.	±3·00	±1·64	±2·02	±1·84
*P**	1 : 2	1 : 2	1 : 2	1 : 1
	Not significant	Not significant	Not significant	Not significant
Body length (mm.):				
Average A, "R" or "S"	192·0	193·0	192·0	193·4
„ B, "P" or "W"	193·0	192·6	192·3	192·1
Difference A – B	−1·0	+0·4	−0·3	+1·3
S.E.M.	±1·33	±1·67	±1·69	±1·56
*P**	1 : 2	1 : 1	1 : 1	1 : 2
	Not significant	Not significant	Not significant	Not significant
Gain per gram solids ingested (g.):				
Average A, "R" or "S"	0·2389	0·2479	0·2408	0·2461
„ B, "P" or "W"	0·2438	0·2509	0·2459	0·2493
Difference A – B	−0·0049	−0·0030	−0·0051	−0·0032
S.E.M.	±0·0064	±0·0034	±0·0043	±0·0040
*P**	1 : 2	1 : 2	1 : 4	1 : 2
	Not significant	Not significant	Not significant	Not significant
Weight/length ratio:				
Average A, "R" or "S"	0·86	0·89	0·87	0·88
„ B, "P" or "W"	0·87	0·89	0·88	0·88
Difference A – B	−0·01	0	−0·01	0
S.E.M.	±0·013	—	±0·026	—
*P**	1 : 2	—	1 : 1	—
	Not significant	—	Not significant	—
% fat in carcass:				
Average A, "R" or "S"	13·48	13·76	13·67	13·50
„ B, "P" or "W"	13·69	14·43	13·70	14·41
Difference A – B	−0·21	−0·67	−0·03	−0·91
S.E.M.	±0·84	±0·49	±0·64	±0·54
P*	1 : 1	1 : 5	1 : 1	1 : 15
	Not significant	Not significant	Not significant	Not significant
% water in carcass:				
Average A, "R" or "S"	63·39	63·28	63·25	63·40
„ B, "P" or "W"	63·23	62·73	63·34	62·77
Difference A – B	+0·16	+0·55	−0·09	+0·63
S.E.M.	±0·62	±0·44	±0·48	±0·45
*P**	1 : 1	1 : 8	1 : 1	1 : 5
	Not significant	Not significant	Not significant	Not significant
% dry fat-free residue in carcass:				
Average A, "R" or "S"	23·13	22·99	23·08	23·16
„ B, "P" or "W"	23·07	22·79	22·96	22·77
Difference A – B	+0·06	+0·20	+0·12	+0·39
S.E.M.	±0·24	±0·20	±0·21	±0·24
*P**	1 : 1	1 : 3	1 : 2	1 : 7
	Not significant	Not significant	Not significant	Not significant

* P = probability that a difference at least as great as the observed difference should have arisen by an accident of sampling from a homogeneous population.

Table IV*a*. *Statistical treatment of the differences in refusals of milk of the experimental rats on exclusive milk diets*

	Summer milk. Raw *v.* pasteurized	"Winter" milk. Raw *v.* pasteurized	Raw milk. Summer *v.* "winter"	Pasteurized milk. Summer *v.* "winter"
No. of animals refusing more frequently on specified milk:				
Total A, "R" or "S"	6·5	4	5	5
,, B, "P" or "W"	4·5	7	7	5
Difference from mean	1	1·5	1	0
Standard error	±1·63	±1·59	±1·71	±1·58
*P**	1 : 2 Not significant	1 : 3 Not significant	1 : 2 Not significant	— Not significant
Total number of refusals on specified milk:				
Total A, "R" or "S"	130	144	130	109
,, B, "P" or "W"	112	163	170	158
Difference from mean	9	9·5	20	24·5
Standard error	±7·76	±8·74	±8·58	±8·03
*P**	1 : 4 Not significant	1 : 3 Not significant	1 : 50 Significant	Over 1 : 100 Significant

* P = probability that a difference at least as great as the observed difference should have arisen by an accident of sampling from a homogeneous population.

Table IV*b*. *Statistical tests of the median quantity (ml.) of milk refused by the experimental rats on exclusive milk diets*†

	Summer raw	Summer pasteurized	"Winter" raw	"Winter" pasteurized	Summer pooled	"Winter" pooled
Measurements which define median	30, 34	29, 32	68, 86	60, 94	30, 32	68, 86
Median quantity of milk rejected (12 rats)	32	30·5	77	77	31	77
Estimated probable error of median. ($\sqrt{\pi/2n}$ × semi-interquartile range)	±13·2	±11·5	±14·0	±25·6	±8·2	±13·2

† These tests are discussed in detail in the accompanying paper by White[16].

The average daily gain in weight for the entire experiment was only about 2 g. This is rather a low figure. All rats showed an initial drop in weight after being removed from the stock diet and placed on milk only. By the end of the first week growth had been resumed and was well established by the end of the second week. The gain during the final 6 weeks was about 2·4 g. per day which although not very good, may be considered satisfactory if it is remembered that the milk intake had to be equalized within groups of four rats.

The average milk intakes were practically identical for all four groups. The small differences which occurred within groups may be accounted for by the fact that one or more rats in a group left milk on the last day. Average figures for body length, for the weight : length ratios and for the gain per gram solids ingested also show very small differences. Statistical tests on all of the above measurements show that the differences are not significant (Table IV).

The analyses of the carcasses for fat, water and dry fat-free residue are given in Table III. In addition to the two groups of rats already mentioned a further group had to be omitted from certain averages because the fat analysis

of one rat (No. 5345) was lost. Statistical tests (Table IV) show that the differences in composition of the carcasses are quite insignificant.

Tables IV*a* and IV*b* give a statistical analysis of the milk refusals. These data are discussed on p. 304.

Rough records of scouring made at the bi-weekly cleaning of the cages indicated that the incidence of scouring was greater on summer milk than on "winter" milk, particularly when it was fed raw.

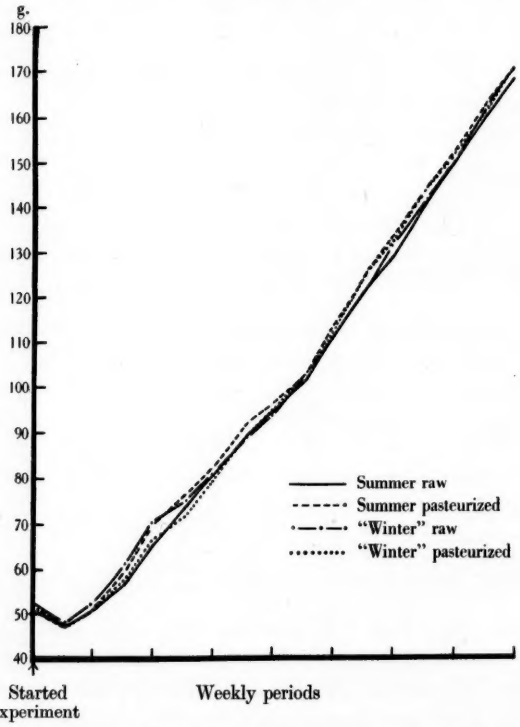


Fig. 1. Average growth curves (12 ♂♂ each) of rats receiving an exclusive diet of summer or "winter" milk before and after pasteurization, supplemented with minerals. Milk intakes equalized within groups of four rats.

There were no noticeable differences in the appearance of the rats belonging to the different groups, when judged by the usual criteria of sleekness of coat, brightness of eye, muscular tonus and "handling".

(*b*) *Milk fed together with a deficient diet.*

When discussing the failure of our previous experiments (3) to disclose any difference between winter raw and winter pasteurized milks when fed as an exclusive diet we suggested that any possible differences would be masked at such high levels of milk intake when all known constituents of milk are

available either in great excess or at least with a good margin of safety. We also believed that our experiments had shown that no unknown essential constituent had been drastically reduced by pasteurization.

The following experiments were therefore designed in which the rats obtained protein, calories and minerals from a basal diet. Milk, in limited quantities, was to supply the other essential constituents.

The basal diet (diet 68) had the following composition:

	%
Casein*	24
Sugar (castor)	·72
Salts (Steenbock's 40)	4

* New Zealand lactic washed six times at the isoelectric point with water acidulated with HCl.

Though carefully washed, the casein probably still contained certain vitamin factors. It seems to us very doubtful, however, whether it could possibly contain enough of the substances said to be found in pasture milk to vitiate the experiment.

These experiments were, in a way, similar to the experiments of Stirn *et al.* (8), in which milk serum supplemented a highly purified diet.

Four groups of four litter-mate does and six groups of four and two groups of three litter-mate bucks were placed in individual cages on screens, when 22–24 days old and weighing 38–62 g. The above diet was offered *ad lib.* in glass dishes under stainless steel hoods as used in metabolic experiments in this laboratory (Henry & Kon (9)). Records of the food consumption were kept. The trays under the cages were lined with white blotting paper, and any food scattered was thus easily recovered. After the first few days only very small amounts were scattered. In addition to the diet each rat was offered at the start of the experiment 10 ml. daily of one of the four experimental milks. If a rat had not consumed all its milk by the following morning the unconsumed milk was measured back and the same amount of fresh milk offered with the day's allowance. As some rats left milk badly at first, the daily allowance was cut down on the fourth day to 5 ml. for all rats. The amount was then increased at the beginning of the second week to 10 ml., at the beginning of the third week to 15 ml. and at the beginning of the fourth week to 20 ml. daily. After a short time all rats cleared their milk quantitatively. No milk was given on Sundays.

At the end of 4 weeks the growth of the rats was not very good (Fig. 2*a*, *b*). As an added precaution the minerals (especially copper), not included as such in the Steenbock's salts, were added to the diet in the form of Avellar de Loureiro's (10) "trace" mixture. Although unlikely, the possibility of a shortage of copper in the diet could not be overlooked, though there was probably sufficient copper present as an impurity in the casein and salts to cover, with the amount present in the milk, the daily requirement of the rat. 0·3 g. of "trace" salt mixture was added per 100 g. of the Steenbock salt mixture (diet 68 A).

There was no improvement in growth after the addition of the minerals. But when after another 4 weeks 5% of brewer's yeast was added to the diet (diet 69), there was a marked increase in the rate of growth (Fig. 2 *a*, *b*). After 2 weeks on this supplemented diet the rats were killed by coal gas and the body length measured in the usual way from the tip of the nose to the centre of the anus. The experiment lasted from 5 May to 15 July 1936.

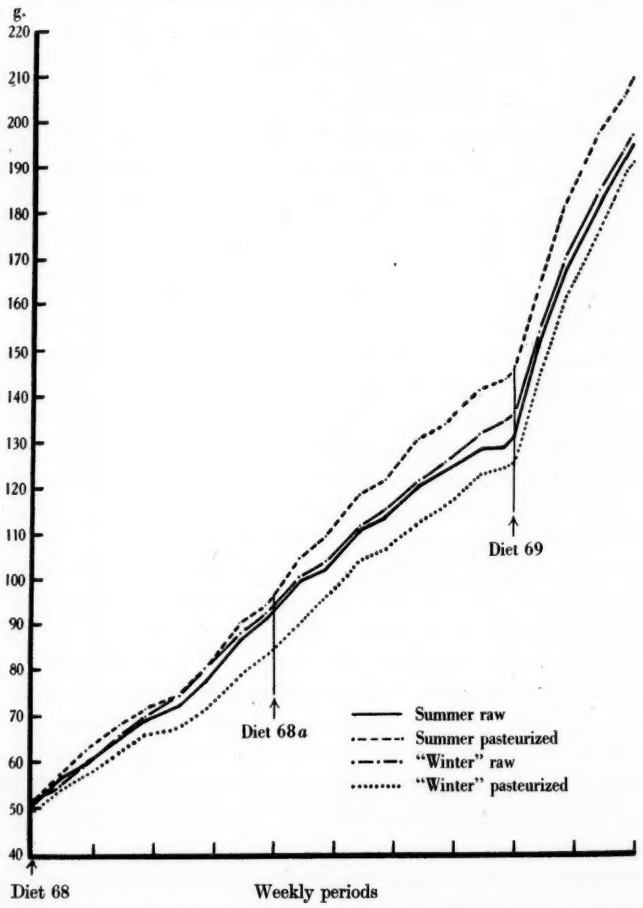


Fig. 2*a*. Average growth curves (7 or 8 ♂♂ each) of male rats receiving a basal diet in addition to a fixed allowance of milk.

The results are shown in Table V (*a* and *b*), which gives the initial and final weights, the gains for the whole period and per day, the intake of food, milk and total solids, and the gain per gram solids ingested for (*a*) the period on the deficient diet and (*b*) the period after the addition of yeast to the diet. Table V*b* also includes the body length of the animals when killed. Table VI (*a* and *b*)

shows the average figures for separate and pooled sexes. The results of the statistical tests are given in Table VII. Growth curves for bucks and does are shown in Fig. 2 *a*, *b* respectively.

It will be seen from these figures that for both sexes the rats on summer pasteurized milk and diet 68 showed better growth than the animals on the other milks, the differences being more marked in the case of the bucks than of the does. After the addition of yeast to the diet the difference decreased. Average figures for growth are shown in Table VI*a*, from which it can be seen

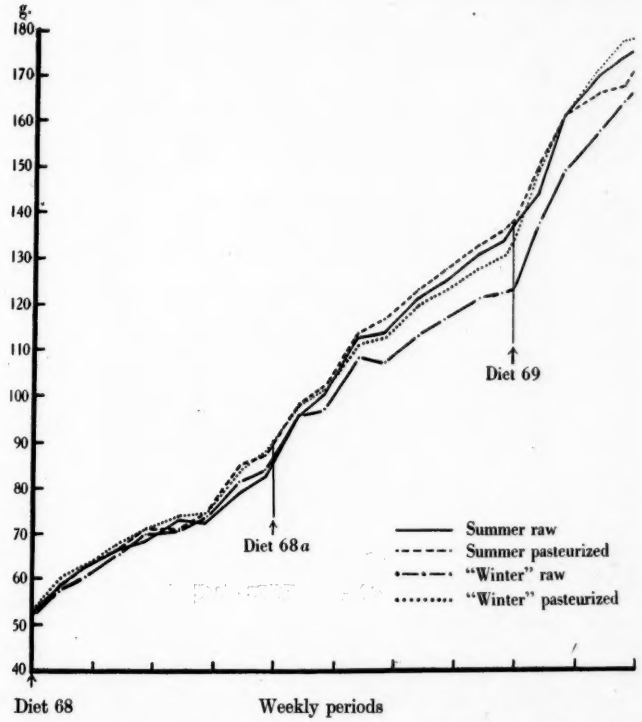


Fig. 2*b*. Average growth curves (4♀♀ each) of female rats receiving a basal diet in addition to a fixed allowance of milk.

that for the pooled sexes the best growth was that of the rats on the summer pasteurized milk, and the poorest that of the rats on "winter" pasteurized milk. Statistical tests (Table VII) show that the growth on summer pasteurized milk is significantly better than that on raw summer milk, the difference in favour of the pasteurized milk being 11·0 g. with a standard error of the mean (S.E.M.) of $\pm 3{\cdot}74$ and a probability (P) of 1/66 that the difference is due to chance. Table VII also shows that the gain on summer pasteurized milk is significantly better than that on "winter" pasteurized, the difference in favour of the summer milk being 13·9 g. with an S.E.M. of $\pm 3{\cdot}12$ and $P = 1/1000$. The

Table V. *Weights, gains in weight, daily gains in weight, intakes of basal diet, milk and total solids, gains per gram solids ingested and body lengths of experimental rats receiving milk in addition to (a) a basal deficient diet (diet 68), (b) a basal deficient diet + 5% yeast (diet 69)*

(*a*) Diet 68

Litter no.	Rat no. and sex	Weight (g.) Initial	Final	Gain	Gain per day g.	Basal diet intake g.	Milk intake ml.	*Total solid intake g.	Gain per gram solids ingested g.	Body length when killed mm.
					Summer raw					
I	5326♀	48	155	107	1·91	428·90	795	520·09	0·2057	—
II*a*	5358♀	62	147	85	1·52	402·58	795	494·35	0·1719	—
III	5369♀	47	118	71	1·27	313·25	795	406·99	0·1745	—
IV	5380♀	56	126	70	1·25	372·53	795	464·96	0·1506	—
V*a*	5374♂	55	143	88	1·57	412·56	795	504·11	0·1746	—
V*b*	5376♂	53	158	105	1·88	409·03	795	500·66	0·2097	—
VI	5406♂	49	110	61	1·09	297·88	795	391·96	0·1556	—
II*b*	—	—	—	—	—	—	—	—	—	—
VII	5442♂	48	122	74	1·32	315·26	795	408·95	0·1810	—
VIII	5465♂	52	142	90	1·61	366·71	795	459·27	0·1960	—
IX	5480♂	50	130	80	1·43	345·62	795	438·65	0·1824	—
X	5449♂	52	109	57	1·02	307·61	795	401·47	0·1420	—
					Summer pasteurized					
I	5322♀	50	151	101	1·80	402·57	795	494·34	0·2043	—
II*a*	5357♀	60	147	87	1·55	405·91	795	497·61	0·1748	—
III	5366♀	47	130	83	1·48	370·63	795	463·11	0·1792	—
IV	5381♀	52	123	71	1·27	372·40	795	464·84	0·1527	—
V*a*	5372♂	52	159	107	1·91	427·51	795	518·73	0·2063	—
V*b*	5377♂	57	172	115	2·05	456·23	795	546·82	0·2103	—
VI	5410♂	44	130	86	1·54	346·67	795	439·67	0·1956	—
II*b*	5362♂	60	142	82	1·46	400·57	795	492·39	0·1665	—
VII	5440♂	52	152	100	1·79	358·73	795	451·46	0·2215	—
VIII	5468♂	51	134	83	1·48	336·59	795	429·82	0·1931	—
IX	5483♂	51	158	107	1·91	410·99	795	502·58	0·2129	—
X	5448♂	50	119	69	1·23	315·66	795	409·35	0·1686	—
					"Winter" raw					
I	5324♀	49	128	79	1·41	357·00	795	448·33	0·1762	—
II*a*	5359♀	60	135	75	1·34	376·73	795	467·62	0·1604	—
III	5368♀	47	119	72	1·29	333·07	795	424·92	0·1694	—
IV	5382♀	53	111	58	1·04	334·19	795	426·01	0·1361	—
V*a*	5373♂	53	156	103	1·84	425·75	795	515·56	0·1998	—
V*b*	5379♂	41	163	122	2·18	402·57	795	492·89	0·2475	—
VI	—	—	—	—	—	—	—	—	—	—
II*b*	5361♂	61	102	41	0·73	341·96	795	433·61	0·0946	—
VII	5438♂	52	122	70	1·25	293·78	795	386·50	0·1811	—
VIII	5469♂	52	124	72	1·29	312·07	795	404·38	0·1781	—
IX	5481♂	57	157	100	1·79	379·33	795	470·16	0·2127	—
X	5447♂	54	127	73	1·30	328·37	795	420·32	0·1737	—
					"Winter" pasteurized					
I	5320♀	48	134	86	1·54	369·97	795	461·01	0·1865	—
II*a*	5360♀	61	141	80	1·43	376·31	795	467·21	0·1712	—
III	5367♀	51	139	88	1·57	411·60	795	501·72	0·1754	—
IV	5383♀	55	118	63	1·13	329·58	795	421·51	0·1495	—
V*a*	5375♂	38	142	104	1·86	375·17	795	466·10	0·2231	—
V*b*	5378♂	44	127	83	1·48	340·16	795	431·86	0·1922	—
VI	5409♂	48	114	66	1·18	298·26	795	390·88	0·1688	—
II*b*	5363♂	62	123	61	1·09	368·85	795	459·92	0·1326	—
VII	5441♂	49	130	81	1·45	324·17	795	416·22	0·1946	—
VIII	5470♂	52	128	76	1·36	355·71	795	447·06	0·1700	—
IX	5482♂	53	131	78	1·39	329·52	795	421·45	0·1851	—
X	5446♂	52	110	58	1·04	298·80	795	391·41	0·1482	—

Table V (*continued*)

(*b*) Diet 69

Litter no.	Rat no. and sex	Weight (g.) Initial	Final	Gain	Gain per day g.	Basal diet intake g.	Milk intake ml.	*Total solid intake g.	Gain per gram solids ingested g.	Body length when killed mm.
					Summer raw					
I	5326♀	155	194	39	2·79	193·62	240	218·95	0·1781	199
II*a*	5358♀	147	194	47	3·36	180·92	240	206·57	0·2275	194
III	5369♀	118	154	36	2·57	141·20	240	167·84	0·2145	187
IV	5380♀	126	157	31	2·21	166·12	240	192·14	0·1613	183
V*a*	5374♂	143	200	57	4·07	208·37	240	233·33	0·2443	201
V*b*	5376♂	158	206	48	3·43	192·62	240	217·97	0·2202	202
VI	5406♂	110	186	76	5·43	183·37	240	208·96	0·3637	196
II*b*	—	—	—	—	—	—	—	—	—	—
VII	5442♂	122	186	64	4·57	168·41	240	194·37	0·3293	197
VIII	5465♂	142	220	78	5·57	212·16	240	237·03	0·3291	206
IX	5480♂	130	196	66	4·71	185·17	240	210·71	0·3132	197
X	5449♂	109	167	58	4·14	163·60	240	189·68	0·3058	196
					Summer pasteurized					
I	5322♀	151	182	31	2·21	162·22	240	188·33	0·1646	195
II*a*	5357♀	147	198	51	3·64	193·13	240	218·47	0·2334	194
III	5366♀	130	155	25	1·79	144·53	240	171·09	0·1461	189
IV	5381♀	123	145	22	1·57	134·31	240	161·12	0·1365	180
V*a*	5372♂	159	211	52	3·71	210·76	240	235·66	0·2207	206
V*b*	5377♂	172	215	43	3·07	187·08	240	212·57	0·2023	209
VI	5410♂	130	199	69	4·93	193·47	240	218·80	0·3154	203
II*b*	5362♂	142	196	54	3·86	177·80	240	203·53	0·2653	201
VII	5440♂	152	223	71	5·07	210·51	240	235·42	0·3016	208
VIII	5468♂	134	207	73	5·21	193·96	240	219·28	0·3329	205
IX	5483♂	158	236	78	5·57	249·34	240	273·28	0·2854	214
X	5448♂	119	186	67	4·79	180·38	240	206·04	0·3252	201
					"Winter" raw					
I	5324♀	128	167	39	2·79	146·27	240	172·78	0·2257	190
II*a*	5359♀	135	176	41	2·93	153·76	240	180·09	0·2277	191
III	5368♀	119	154	35	2·50	137·13	240	163·87	0·2136	187
IV	5382♀	111	166	55	3·93	162·42	240	188·53	0·2917	191
V*a*	5373♂	156	218	62	4·43	219·89	240	244·56	0·2535	208
V*b*	5379♂	163	209	46	3·29	186·74	240	212·24	0·2167	206
VI	—	—	—	—	—	—	—	—	—	—
II*b*	5361♂	102	164	62	4·43	162·80	240	188·90	0·3282	187
VII	5438♂	122	194	72	5·14	185·18	240	210·72	0·3417	199
VIII	5469♂	124	182	58	4·14	171·18	240	197·07	0·2943	197
IX	5481♂	157	219	62	4·43	207·34	240	232·33	0·2669	209
X	5447♂	127	190	63	4·50	195·06	240	220·35	0·2859	203
					"Winter" pasteurized					
I	5320♀	134	183	49	3·50	182·37	240	207·98	0·2356	195
II*a*	5360♀	141	178	37	2·64	177·00	240	202·75	0·1825	192
III	5367♀	139	180	41	2·93	183·54	240	209·12	0·1961	195
IV	5383♀	118	170	52	3·71	170·37	240	196·28	0·2649	194
V*a*	5375♂	142	192	50	3·57	192·22	240	217·58	0·2298	194
V*b*	5378♂	127	194	67	4·79	194·99	240	220·29	0·3041	197
VI	5409♂	114	187	73	5·21	187·86	240	213·33	0·3422	194
II*b*	5363♂	123	190	67	4·79	176·18	240	201·95	0·3334	196
VII	5441♂	130	204	74	5·29	205·44	240	230·47	0·3211	201
VIII	5470♂	128	180	52	3·71	164·32	240	190·38	0·2731	199
IX	5482♂	131	204	73	5·21	197·32	240	222·56	0·3280	205
X	5446♂	110	173	63	4·50	176·84	240	202·59	0·3110	197

* Calculated on dry matter: solids of milk calculated from the data of Table I on the assumption that the specific gravity of milk is 1·03; moisture diet 68 = 2·24%; moisture diet 69 = 2·50%.

Table VI. *Group averages of the data of Table V*

(*a*) Diet 68

Average no.	Weight (g.) Initial	Final	Gain	Gain per day g.	Basal diet intake g.	Milk intake ml.	Total solid intake g.	Gain per gram solids ingested g.	Body length when killed mm.
				Summer raw					
I	—	—	—	—	—	—	—	—	—
II	52·00	132·73	80·73	1·44	361·08	795	453·77	0·1767	—
III	—	—	—	—	—	—	—	—	—
IV	52·30	135·00	82·70	1·48	367·41	795	459·95	0·1788	—
V	53·25	136·50	83·25	1·49	379·32	795	471·60	0·1757	—
VI	—	—	—	—	—	—	—	—	—
VII	51·29	130·57	79·29	1·42	350·67	795	443·58	0·1773	—
VIII	—	—	—	—	—	—	—	—	—
IX	51·67	134·00	82·33	1·47	359·47	795	452·19	0·1800	—
				Summer pasteurized					
I	52·17	143·08	90·92	1·62	383·71	795	475·89	0·1905	—
II	51·45	143·18	91·73	1·64	382·17	795	474·39	0·1927	—
III	52·91	144·27	91·36	1·63	387·07	795	479·19	0·1900	—
IV	52·20	144·50	92·30	1·65	385·72	795	477·87	0·1924	—
V	52·25	137·75	85·50	1·53	387·88	795	479·98	0·1778	—
VI	52·13	145·75	93·63	1·67	381·62	795	473·85	0·1969	—
VII	51·00	146·29	95·29	1·70	378·91	795	471·20	0·2012	—
VIII	53·29	148·00	94·71	1·69	386·61	795	478·74	0·1970	—
IX	52·17	149·00	96·83	1·73	384·29	795	476·46	0·2021	—
				"Winter" raw					
I	—	—	—	—	—	—	—	—	—
II	—	—	—	—	—	—	—	—	—
III	52·64	131·27	78·64	1·41	353·17	795	444·57	0·1754	—
IV	51·80	134·20	82·40	1·47	354·29	795	445·67	0·1835	—
V	52·25	123·25	71·00	1·27	350·25	795	441·72	0·1605	—
VI	—	—	—	—	—	—	—	—	—
VII	—	—	—	—	—	—	—	—	—
VIII	52·86	135·86	83·00	1·48	354·83	795	446·20	0·1839	—
IX	51·50	141·50	90·00	1·61	356·98	795	448·30	0·1988	—
				"Winter" pasteurized					
I	51·08	128·08	77·00	1·38	348·18	795	439·70	0·1748	—
II	50·09	128·55	78·45	1·40	346·30	795	437·86	0·1786	—
III	51·36	129·36	78·00	1·39	352·71	795	444·13	0·1753	—
IV	50·30	130·00	79·70	1·43	351·10	795	442·56	0·1796	—
V	53·75	133·00	79·25	1·42	371·87	795	462·86	0·1707	—
VI	49·75	125·63	75·88	1·36	336·33	795	428·11	0·1768	—
VII	48·00	126·00	78·00	1·39	331·68	795	423·57	0·1831	—
VIII	50·00	127·29	77·29	1·38	341·77	795	433·43	0·1780	—
IX	48·00	128·00	80·00	1·43	337·26	795	429·02	0·1855	—

Table VI (*continued*)

(*b*) Diet 69

Average no.	Weight (g.) Initial	Final	Gain	Gain per day g.	Basal diet intake g.	Milk intake ml.	Total solid intake g.	Gain per gram solids ingested g.	Body length when killed mm.
				Summer raw					
I	—	—	—	—	—	—	—	—	—
II	132·73	187·27	54·55	3·90	181·41	240	207·05	0·2625	196·18
III	—	—	—	—	—	—	—	—	—
IV	135·00	187·40	52·40	3·74	181·22	240	206·86	0·2523	196·20
V	136·50	174·75	38·25	2·73	170·47	240	196·38	0·1954	190·75
VI	—	—	—	—	—	—	—	—	—
VII	130·57	194·43	63·86	4·56	187·67	240	213·15	0·3008	199·29
VIII	—	—	—	—	—	—	—	—	—
IX	134·00	195·83	61·83	4·42	188·39	240	213·85	0·2903	199·83
				Summer pasteurized					
I	143·08	196·08	53·00	3·79	186·46	240	211·97	0·2441	200·42
II	143·18	196·09	52·91	3·78	187·24	240	212·73	0·2422	200·36
III	144·27	195·82	51·55	3·68	185·82	240	211·34	0·2376	200·18
IV	144·50	195·80	51·30	3·66	186·62	240	212·13	0·2349	200·10
V	137·75	170·00	32·25	2·30	158·55	240	184·75	0·1702	189·50
VI	145·75	209·13	63·38	4·53	200·41	240	225·57	0·2811	205·88
VII	146·29	211·00	64·71	4·62	203·64	240	228·72	0·2834	206·57
VIII	148·00	210·57	62·57	4·47	201·40	240	226·54	0·2762	206·29
IX	149·00	213·00	64·00	4·57	205·34	240	230·38	0·2780	207·17
				"Winter" raw					
I	—	—	—	—	—	—	—	—	—
II	—	—	—	—	—	—	—	—	—
III	131·27	185·36	54·09	3·86	175·25	240	201·04	0·2678	197·09
IV	134·20	187·50	53·30	3·81	176·50	240	202·25	0·2618	198·10
V	123·25	165·75	42·50	3·04	149·90	240	176·32	0·2397	189·75
VI	—	—	—	—	—	—	—	—	—
VII	—	—	—	—	—	—	—	—	—
VIII	135·86	196·57	60·71	4·34	189·74	240	215·17	0·2839	201·29
IX	141·50	202·00	60·50	4·32	194·23	240	219·55	0·2765	203·67
				"Winter" pasteurized					
I	128·08	186·25	58·17	4·15	184·04	240	209·61	0·2768	196·58
II	128·55	185·91	57·36	4·10	184·75	240	210·30	0·2717	196·64
III	129·36	186·18	56·82	4·06	183·69	240	209·27	0·2709	196·82
IV	130·00	185·80	55·80	3·99	184·44	240	210·00	0·2646	196·90
V	133·00	177·75	44·75	3·20	178·32	240	204·03	0·2198	194·00
VI	125·63	190·50	64·88	4·63	186·90	240	212·39	0·2781	197·88
VII	126·00	190·57	64·57	4·61	188·43	240	213·89	0·2702	198·14
VIII	127·29	191·00	63·71	4·55	186·76	240	212·26	0·2689	198·43
IX	128·00	191·17	63·17	4·51	188·52	240	213·98	0·2582	198·83

Average I =average for 12 animals (♀♀ and ♂♂).
Average II =average for 11 animals (♀♀ and ♂♂), omitting litter No. II *b*.
Average III =average for 11 animals (♀♀ and ♂♂), omitting litter No. VI.
Average IV =average for 10 animals (♀♀ and ♂♂), omitting litters Nos. II*b* and VI.
Average V =average for 4 animals (♀♀).
Average VI =average for 8 animals (♂♂).
Average VII =average for 7 animals (♂♂), omitting litter No. II*b*.
Average VIII =average for 7 animals (♂♂), omitting litter No. VI.
Average IX =average for 6 animals (♂♂), omitting litters Nos. II*b* and VI.

Table VII. *Statistical treatment of average differences in gain in weight, basal diet intake, gain per gram solids ingested and body length of rats receiving milk in addition to (a) diet 68, (b) diet 69*

Difference tested	Summer milk. Raw *v.* pasteurized	"Winter" milk. Raw *v.* pasteurized	Raw milk. Summer *v.* "winter"	Pasteurized milk. Summer *v.* "winter"
(*a*) Diet 68				
Gain in weight (g.), ♀♀ and ♂♂:				
Average A, "R" or "S"	80·7	78·6	82·7	90·9
,, B, "P" or "W"	91·7	78·0	82·4	77·0
Difference A – B	−11·0	+ 0·6	+ 0·3	+13·9
S.E.M.	± 3·74	± 5·32	± 5·31	± 3·12
*P**	1 : 67 Significant	1 : 1 Not significant	1 : 1 Not significant	1 : 1000 Significant
Basal diet intake (g.), ♀♀ and ♂♂:				
Average A, "R" or "S"	361·08	353·17	367·41	383·71
,, B, "P" or "W"	382·17	352·71	354·29	348·18
Difference A – B	−21·09	+ 0·46	+13·12	+35·53
S.E.M.	±10·04	±13·39	±11·16	±11·79
*P**	1 : 16 Not significant	1 : 1 Not significant	1 : 4 Not significant	1 : 85 Significant
Gain per gram solids ingested (g.), ♀♀ and ♂♂:				
Average A, "R" or "S"	0·1767	0·1754	0·1788	0·1905
,, B, "P" or "W"	0·1927	0·1753	0·1835	0·1748
Difference A – B	− 0·0160	+ 0·0001	− 0·0047	+ 0·0157
S.E.M.	± 0·0054	± 0·0081	± 0·0077	± 0·0042
*P**	1 : 74 Significant	1 : 1 Not significant	1 : 2 Not significant	1 : 294 Significant
Gain per gram solids ingested (g.), ♀♀:				
Average A, "R" or "S"	0·1757	0·1605	0·1757	0·1778
,, B, "P" or "W"	0·1778	0·1707	0·1605	0·1707
Difference A – B	− 0·0021	− 0·0102	+ 0·0152	+ 0·0071
S.E.M.	± 0·0013	± 0·0015	± 0·0052	± 0·0036
*P**	1 : 5 Not significant	1 : 135 Significant	1 : 17 Not significant	1 : 7 Not significant
Gain per gram solids ingested (g.), ♂♂:				
Average A, "R" or "S"	0·1773	0·1839	0·1809	0·1969
,, B, "P" or "W"	0·2012	0·1780	0·1988	0·1768
Difference A – B	− 0·0239	+ 0·0059	− 0·0179	+ 0·0201
S.E.M.	± 0·0067	± 0·0124	± 0·0089	± 0·0055
*P**	1 : 83 Significant	1 : 1 Not significant	1 : 10 Not significant	1 : 119 Significant
(*b*) Diet 69				
Gain in weight (g.), ♀♀ and ♂♂:				
Average A, "R" or "S"	54·6	54·1	52·4	53·0
,, B, "P" or "W"	52·9	56·8	53·3	58·2
Difference A – B	+ 1·7	− 2·7	− 0·9	− 5·2
S.E.M.	± 1·43	± 2·78	± 3·56	± 4·26
*P**	1 : 2 Not significant	1 : 3 Not significant	1 : 1 Not significant	1 : 4 Not significant
Basal diet intake (g.), ♀♀ and ♂♂:				
Average A, "R" or "S"	181·41	175·25	181·22	186·46
,, B, "P" or "W"	187·24	184·69	176·50	184·04
Difference A – B	− 5·83	− 9·44	+ 4·72	+ 2·42
S.E.M.	± 8·75	± 8·34	± 8·43	± 7·53
*P**	1 : 2 Not significant	1 : 3 Not significant	1 : 1 Not significant	1 : 1 Not significant

Table VII (*continued*)

Difference tested	Summer milk. Raw *v.* pasteurized	"Winter" milk. Raw *v.* pasteurized	Raw milk. Summer *v.* "winter"	Pasteurized milk. Summer *v.* "winter"
Gain per gram solids ingested (g.), ♀♀ and ♂♂:				
Average A, "R" or "S"	0·2625	0·2678	0·2523	0·2441
„ B, "P" or "W"	0·2422	0·2709	0·2618	0·2768
Difference A – B	+ 0·0203	– 0·0031	– 0·0095	– 0·0327
S.E.M.	± 0·0074	± 0·0123	± 0·0158	± 0·0164
*P**	1 : 46 Significant	1 : 1 Not significant	1 : 2 Not significant	1 : 14 Not significant
Body length (mm.), ♀♀ and ♂♂:				
Average A, "R" or "S"	196·2	197·1	196·2	200·4
„ B, "P" or "W"	200·4	196·8	198·1	196·6
Difference A – B	– 4·2	+ 0·3	– 1·9	+ 3·8
S.E.M.	± 1·86	± 2·17	± 2·26	± 2·19
*P**	1 : 20 Significant	1 : 1 Not significant	1 : 2 Not significant	1 : 10 Not significant

* *P* = probability that a difference at least as great as the observed difference should have arisen by an accident of sampling from a homogeneous population.

Note to Table VII. The comparisons between gains per gram of solids ingested by does on summer and "winter" milk indicate differences in favour of summer milk, but the levels of significance (1 : 17 and 1 : 7) are below the accepted level (1 : 20) when data for raw and pasteurized milk are considered separately. On pooling the data, however, there is a marked increase in the level of significance which rises to 1 : 100.

Table VIII. *Average daily intakes of diets* 68 *and* 69

Summer raw			Summer pasteurized			"Winter" raw			"Winter" pasteurized		
Rat no. and sex	Diet intake (g.)		Rat no. and sex	Diet intake (g.)		Rat no. and sex	Diet intake (g.)		Rat no. and sex	Diet intake (g.)	
	68	69		68	69		68	69		68	69
5326♀	7·66	13·83	5322♀	7·19	11·59	5324♀	6·37	10·45	5320♀	6·61	13·03
5358♀	7·19	12·92	5357♀	7·25	13·80	5359♀	6·73	10·98	5360♀	6·72	12·64
5369♀	5·59	10·09	5366♀	6·62	10·32	5368♀	5·95	9·80	5367♀	7·37	13·11
5380♀	6·65	11·87	5381♀	6·65	9·59	5382♀	5·97	11·60	5383♀	5·89	12·17
5374♂	7·37	14·88	5372♂	7·63	15·05	5373♂	7·60	15·71	5375♂	6·70	13·73
5376♂	7·30	13·76	5377♂	8·15	13·36	5379♂	7·19	13·34	5378♂	6·07	13·93
5406♂	5·32	13·10	5410♂	6·19	13·82				5409♂	5·33	13·42
			5362♂	7·15	12·70	5361♂	6·11	11·63	5363♂	6·59	12·58
5442♂	5·63	12·03	5440♂	6·41	15·04	5438♂	5·25	13·23	5441♂	5·79	14·67
5465♂	6·55	15·15	5468♂	6·01	13·85	5469♂	5·57	12·23	5470♂	6·35	11·74
5480♂	6·17	13·23	5483♂	7·34	17·81	5481♂	6·77	14·81	5482♂	5·88	14·09
5449♂	5·49	11·69	5448♂	5·64	12·88	5447♂	5·86	13·93	5446♂	5·34	12·63
Av. ♀♀ and ♂♂	6·45	12·96		6·85	13·32		6·31	12·52		6·22	13·15
Av. ♀♀	6·77	12·18		6·92	11·32		6·25	10·71		6·65	12·74
Av. ♂♂	6·26	13·41		6·81	14·31		6·34	13·56		6·01	13·35

differences between raw milks, whether summer or "winter", and between "winter" milks, whether raw or pasteurized, are without significance.

While each animal received an equal amount of milk during the course of the experiment (namely 795 ml.), the intakes of diet differed as they were governed by the appetite of the animal (Tables VI and VIII). But a comparison (Table VII) shows that these differences between corresponding averages are

mostly without statistical significance. Thus rats on "winter" milk took almost the same quantities of diet whether the milk was pasteurized or raw. It is most probably due to chance (odds of 1 : 4) that rats on raw summer milk took more diet than those on raw "winter" milk. The difference is more nearly significant (16 to 1 against chance factors) when diet intakes on summer milk before and after pasteurization are compared, but the difference is in favour of pasteurized milk. The only difference which is definitely statistically significant is that between pasteurized summer and pasteurized "winter" milk.

It might be expected that when the gain in weight per gram of solids ingested is calculated the difference in gains of weight would tend to disappear, but this is not the case. The significance of the superior gain of the group on pasteurized over that on raw summer milk increases slightly ($P = 1/74$ as against 1/67) when calculated per gram of solids intake, while the significance of the superior gain on summer milk over "winter" milk when pasteurized decreases from 1/1000 to 1/294 but remains significant. This change in significance is probably accounted for by the fact that while the food intake of the rats on summer pasteurized milk was not greater than that of rats on raw summer milk, it was definitely better than that of rats on "winter" pasteurized milk. Turning to the figures for separate sexes it will be seen that the superior growth of rats on summer pasteurized milk over those on summer raw or "winter" pasteurized is only significant in the case of the faster growing males. The only significant difference for does is shown by the superior growth of the animals on pasteurized over raw "winter" milk.

The daily gain in weight of all the animals was poor, averaging only about 1·5 g. per day. It has been previously shown by Kon (11) that 16–20 ml. of milk daily is sufficient to produce "normal" growth when fed in addition to a vitamin B-deficient diet containing gelatinized potato starch. In the present experiment sugar was given as the source of carbohydrate. It is generally known that the requirements of the rat for the vitamin B complex are higher on diets containing sugar than on starch diets. We thought, therefore, that the poor growth of our rats might be due to the lack of one or more component of the vitamin B complex, a supposition which was borne out by the fact that vastly improved growth occurred on the addition of 5% brewer's yeast. The average growth in the course of 2 weeks increased to nearly 4 g. daily, while Table VIII shows that the daily food intake was approximately doubled during this period. The differences in gain in weight of the rats together with other data are shown in Table V*b*, while the average figures will be found in Table VI*b*.

It will be seen that the differences in gain in weight between the groups have become much smaller, and Table VII shows that they are now without any statistical significance. When the differences in the gain in weight per gram of solids ingested are tested statistically, the summer milk is found to have stimulated better growth when fed raw than after pasteurization (the

probability that this difference is due to chance being 1 : 46), other differences are without statistical significance. There were no significant differences in the intake of basal diet between the different groups.

Discussion

Our experiments with exclusive milk feeding do not show any differences between the milks, due either to season (summer or winter feeding of the cows) or to the treatment of the milk (whether raw or pasteurized) when judged by criteria of gain in weight, body length, composition of the carcasses and appearance of the rats on equalized milk intakes. There seemed to be, however, more tendency to scouring on the raw summer milk than the other milks. The main findings are in complete agreement with our earlier experience (Henry & Kon (3)) and also with that of Krauss (12) who, in paired feeding experiments, found no difference between the growth of rats on raw and pasteurized milks from the same bulk measured at different times of the year, or between the growths of rats on "dry feed" and pasture milks when compared at the same time. As far as these findings go they do not confirm the views of Elvehjem *et al.* (2). The techniques are, however, not comparable, as their experiments were carried out under conditions of *ad lib.* feeding. Under such conditions Krauss (12) also failed to detect a difference between pasture and "dry-feed" milk when the gains per 100 ml. of milk were considered. He does not state the total gains of his animals, so that a direct comparison with the findings of Elvehjem *et al.* (2) is not possible. But it seems clear, especially in view of information kindly supplied by Prof. Hart (13), that the differences in gain in weight between the experimental groups of the latter authors are not due to differences in the efficiency of utilization of the milks but to differences of appetite.

The relative merits of experiments in which the food intakes are equalized and of those where the diet is fed *ad lib.* have recently been ably summarized by Brody (14) and by Johnson *et al.* (15). It cannot be disputed that a dietary deficiency may neither affect the utilization of energy nor the composition of the gains in weight and yet have a definite effect on the appetite of the animal. In such case an *ad lib.* feeding experiment will easily indicate a difference between the deficient and the satisfactory diet, while a paired feeding experiment in which only growth and composition of gains are measured may fail to do so.

In dealing with the results of such a paired feeding experiment it is, therefore, essential to have some criterion for determining whether the data show any evidence of a significant difference in appetite. This question is dealt with by White (16) in an accompanying paper.

It will be remembered that Henry & Kon (3), in experiments on the effects of commercial pasteurization, found no significant differences in the appetites of the rats on the raw and heated milks and were hence confident that their results did not support, in this respect, the work of Elvehjem and his colleagues.

The present experiment likewise gives no indication of an untoward effect of pasteurization on the appetite of the animals. This is evident from Tables IV*a*, *b*.

These tables embody the results of three distinct statistical tests which are discussed in the accompanying paper (16). The first test (Table IV*a*, upper) applies to the numbers of rats refusing a specified milk more frequently and is shown (16) to underestimate the level of significance. The second (Table IV*a*, lower) applies to the total number of refusals of each type of milk and overestimates the level of significance (16). The third test (Table IV*b*) deals with the median quantity refused and probably lies between the other two in sensitiveness. None of these tests reveals any significant difference between raw and pasteurized milk whether produced under summer or "winter" conditions.

Applying the same tests to the comparison between summer and "winter" milk we find that the first test reveals no significant difference whether the milk is raw or pasteurized. The second test appears to indicate that summer milk is more palatable than "winter" milk in both cases, but in view of the known tendency of this test to exaggerate the level of significance, it is not desirable to assign too much weight to this finding. The third test does not support these conclusions when applied to the data for raw and pasteurized milk separately, but when applied to the pooled data reveals a significantly greater palatability in the summer milk.

It would appear therefore that if our animals were allowed *ad lib.* access to milk, those on pasture milk might have made better growth, which would support the findings of Elvehjem *et al.* (2). This would suggest that a substance essential to adequate nutrition is not present in sufficient concentration in the "winter" milk, and is the limiting factor even when the milk is consumed at the high level of almost 70 ml. daily. One would expect that by cutting down the intake of milk any differences between the various milks would tend to increase.

For this reason we carried out the experiments in which the rats were given limited quantities of milk in addition to a diet which we believed supplied little or nothing beyond protein, energy and minerals. Should the appetite of the animals be stimulated to a varying degree by the different milks, the effect could be easily measured by differences in intake of the basal diet, and a difference in gains in weight.

The results of this series of experiments offer no evidence in favour of the superiority of pasture milk over milk from stall-fed cows or of raw milk over pasteurized. As a matter of fact the only significant (or almost significant) differences in these comparisons are in favour of pasteurized summer milk when compared with summer raw, and in favour of pasteurized "winter" milk against raw when the does only are considered. Admittedly we found statistically a marked difference in favour of summer milk as compared with "winter" milk when both were pasteurized;[1] but the difference was not so much due to

[1] Another significant difference for pooled data is given in a footnote to Table VII.

an impairment of the "winter" milk on heating as to the unexpected improvement in summer milk after pasteurization. The interpretation of such findings is not easy.

The very marked increase in food consumption (and gain in weight) of all groups of rats following the addition of 5% brewer's yeast to the basal diet clearly shows that the milks did not supply enough of some essential factor carried by the yeast and most probably belonging to the vitamin B complex. In this respect summer milk appears to be no better than "winter" and pasteurized milk no worse than raw.

This experiment does not bear out our expectation that by limiting the intake we could demonstrate differences between the various types of milk.

To sum up: considered together, the two sets of experiments, one with equalized milk intakes, the other where a supplementary diet was fed, have failed in our hands, we believe, to demonstrate differences in total nutritive value between milk produced on pasture and milk produced under stall-feeding conditions whether these were raw or pasteurized.

SUMMARY

Milk was obtained simultaneously from cows on early pasture and from stall-fed cows receiving winter rations. By suitable blending of the morning's and evening's milkings of each milk the fat content of the two milks was equalized daily. A part of each milk was then pasteurized in the laboratory by a "holder" method. The total nutritive value of the milk was measured on rats in two separate experiments. In one the milks, supplemented with iron, copper and manganese, were fed as an exclusive diet. The four types of milk were given to twelve groups of litter-mate male rats, the intake being equalized within each group. The experiment lasted 8 weeks, and at the end no difference was found in gain in weight, body length, general appearance of the rats or composition of the carcasses. The palatability of the milks as gauged by the refusals of the rats was investigated by various statistical methods, which showed that summer milk was probably more palatable than "winter" milk, but that pasteurization had no effect.

In the second experiment the intake of milk was limited to 20 ml. daily, but the rats were given in addition unlimited access to a basal diet of casein, sugar and salts. This experiment was also carried out on groups of four litter mates (four groups of does and seven groups of bucks). After 8 weeks 5% brewer's yeast was added to the basal diet, resulting in a marked increase of the growth rate of all the groups. At the end of the 8 weeks, the gains in weight, intakes of basal diet, and gains per gram of solids ingested were compared. The only statistically significant differences were in favour of pasteurized summer milk when compared with summer raw and with "winter" pasteurized.

Taking both experiments into consideration it is concluded that they did not reveal any differences in the total nutritive value of the milks and thus did

not demonstrate the presence in pasture or raw milks of new essential factors in addition to those already well known to vary with the season or to be affected by pasteurization.

ACKNOWLEDGEMENTS

We are very much indebted to Miss D. V. Dearden and Miss Salt for blending the experimental milks, and to the former and Mr W. A. Hoy for advice on the pasteurizer. Mr F. K. Neave kindly carried out the phosphatase tests.

REFERENCES

(1) HENRY & KON (1936). *J. Soc. chem. Ind.* **55**, 839.
(2) ELVEHJEM, HART, JACKSON & WECKEL (1934). *J. Dairy Sci.* **17**, 763.
(3) HENRY & KON (1937 *a*). *Milk and Nutrition*, Part I, p. 57. Reading: Nat. Inst. Res. Dairying.
(4) CAMPION, HENRY, KON & MACKINTOSH (1937). *Biochem. J.* **31**, 81.
(5) KAY & GRAHAM (1935). *J. Dairy Res.* **6**, 191.
(6) DONALDSON (1924). *The Rat*, 2nd ed. p. 202. Philadelphia: Wistar Inst. Anat. Biol.
(7) WHITE (1937). *Milk and Nutrition*, Part I, p. 64. Reading: Nat. Inst. Res. Dairying.
(8) STIRN, ELVEHJEM & HART (1935). *J. Dairy Sci.* **18**, 333.
(9) HENRY & KON (1937 *b*). *Milk and Nutrition*, Part I, p. 9. Reading: Nat. Inst. Res. Dairying.
(10) AVELLAR DE LOUREIRO (1931). *Arch. Patol. Lisboa*, **3**, 72.
(11) KON (1933). Unpublished results.
(12) KRAUSS (1936). *Bull Ohio agric. Exp. Sta.* No. 561, pp. 76, 78.
(13) HART (1936). Private communication.
(14) BRODY (1935). *Ann. Rev. Biochem.* **4**, 383.
(15) JOHNSON, HOGAN & ASHWORTH (1936). *Res. Bull. Mo. agric. Exp. Sta.* No. 246.
(16) WHITE (1937). *J. Dairy Res.* **8**, 307.

(*MS. received for publication* 22 *June* 1937)

578.082.2:579.2

165. THE STATISTICAL TREATMENT OF EXPERIMENTS INVOLVING EQUALIZED FEEDING

THE PROBLEM OF APPETITE

By PAUL WHITE
University of Reading

In an *ad libitum* feeding experiment the greater growth of a group of animals may be due either to superior availability or to superior palatability of their diet. To distinguish between these two factors it is necessary to eliminate the effect of superior palatability by equalizing the food intake of corresponding animals. If the quantity offered is so small that it is always consumed entirely, the range of application of the experiment is restricted to conditions of sub-starvation, and at the same time the possibility of obtaining from the results any evidence of difference of palatability is sacrificed. The ration plan must therefore be designed to be as liberal as possible subject to the conditions that the aggregate food intakes are equalized in pairs (or larger groups), while at the same time the animal with the smallest appetite of each pair (or group) is always on the border-line of refusing a little of its portion. In the first set of experiments described in the preceding paper(1) a system of this kind is employed.

The questions then arise as to how the records of refusals can be made to furnish evidence of differences in palatability between the different types of foodstuffs, and how the level of significance of any observed difference is to be tested.

The crudest method, applicable only to cases in which there is a marked difference in appetite, is to consider the number of animals which, when compared each with its group mates, either refused a greater total quantity of food or refused on a greater number of days. The latter basis of comparison is used in the upper entries of Table IV*a* of the preceding paper(1). Taking the entries of col. 3 as an example, we find that in five groups of litter-mates the rat on raw summer milk refused more frequently than that on raw "winter" milk, while in seven groups the opposite occurred. If there were no actual difference in palatability, the most probable result would be that six groups should fall in each class, but the difference of unity in either side is not surprising on account of the variability in appetite inherent in the animals themselves. To test the significance of the difference we note that the distribution of inherent appetite between the groups is a series of independent events, and hence that the formulae appropriate to the tossing of coins apply. Using the binomial distribution we can easily show that the odds (P) are only

1 : 1·29 against the occurrence of an arrangement at least as uneven as 5 and 7, and that the observed result is therefore quite likely to have arisen by chance and cannot be taken as evidence of a significant difference in palatability between the milks. The odds quoted in Table IV*a* were actually calculated from the normal distribution towards which the binomial distribution tends for large numbers. This approximation is clearly sufficient when it gives a very definite judgement on the question of significance, but would have to be replaced by the more accurate formula for borderline cases.

The method just described takes no account of the extent of each rat's refusal, and thus, by rejecting part of the available data, will frequently fail to reveal differences in palatability which a more sensitive method might detect. An obvious refinement is to consider the total number of days on which each rat refused. This method is worked out in the lower part of Table IV*a*. Here the numbers to be compared are 130 (days of refusal of raw summer milk) and 170 (days of refusal of raw "winter" milk). These numbers are large enough to justify the use of the formulae for the normal distribution (2), but the method is open to the objection that the individual refusals are not independent events. The fact that a rat has refused on one day makes it more likely that it will refuse the next day, and as a result the prominence of the differences will be exaggerated. This method therefore provides an upper limit to the level of significance of the data just as the previous one provides a lower limit.

If the data show a strongly significant result on the basis of number of rats (method I) or a non-significant result on the basis of number of separate refusals (method II) the position is plain, but in many cases it is method I which fails to show significance while method II gives an apparently significant result, and we do not know to what extent the latter must be discounted. What is needed is obviously a method which will make use of the *amounts* of food refused. The difficulty here is that the data for the total amounts refused by each rat (Table II*b*) show a markedly non-normal distribution. A few animals fell into a condition in which they steadily refused over long periods and finally contributed figures, disproportionately greater than the others. Indeed, in two cases, indicated by an asterisk, it became obvious that the other litter-mates were being deprived of large amounts of milk which they would have been able to consume, had their ration not been limited by the appetite of the exceptional animal and it became necessary to rearrange the ration plan without regard to the latter.

On account of this unsymmetrical distribution it would be misleading to use the arithmetical means of the quantities refused on each type of food as measures of palatability. In such a case two lines of attack are open. Either we may take the median as a measure or we may transform mathematically the quantity refused so that the distribution of the transformed quantity is approximately normal. In the latter case the usual tests for significance can be applied to the transformed quantity (3).

The medians are given in Table IV*b* of the preceding paper (1). The most convenient estimate of the probable error of the median is $\sqrt{\pi/2n}$ times the semi-interquartile range, where n is the number of measurements. This quantity is listed in the second row of the same table.

The median for winter raw milk exceeds that for summer raw milk by 45 ml. with a probable error which we may take to be $\sqrt{(14^2+13{\cdot}2^2)}$ or $\pm 19{\cdot}2$ ml. A difference of 2·35 times the probable error cannot be taken as conclusive (P=about 1 : 9) but provides an indication well worth following up, especially since on pasteurized milk there is a difference (in the same sense) of 1·65 times the probable error ($P=1:4$). We accordingly examine the pooled (raw and pasteurized) data and find a difference of 46 ml. with probable error of $\pm 15{\cdot}5$ ml. ($P=1:22$). It appears therefore that "winter" milk is significantly less palatable than summer milk.

The median test which ascribes no weight whatever to the outlying numerical values fails to reveal any significant effect of pasteurization on palatability, but inspection of the outlying values gives the impression that it is just among these that pasteurization makes itself felt. In particular the two rats which refused so regularly that they were excluded from the ration plan were both on pasteurized milk, while the only two rats which completely consumed their portion throughout the experiment were on raw milk. The requisite test is clearly one which takes every measurement into account and at the same time overcomes the non-normality of the distribution. We can obtain such a test by working not with the measured total volume refused by each rat but with the quantity, Q, related to it by the equation

$$Q = \text{logarithm } (3 + \text{number of ml. refused}),$$

and examining the significance of any difference in the mean value of Q by the usual methods, e.g. the t-test (3). This form for the function, Q, has no theoretical basis but has been chosen in the light of the distribution of the data actually under consideration. In particular the value of the additive term, 3, which is necessary in any case in order to avoid the occurrence of an absurd value of $Q\,(-\infty)$ when the quantity refused is nil, has been chosen by trial so as to make the distribution of Q symmetrical. It does, in fact, make the distribution very nearly normal in the present instance.

The application of the t-test (3) to the mean values of Q corresponding to the refusals on raw and pasteurized milk shows no significant difference, and we may therefore conclude that in this case any effect of pasteurization on palatability is too small to be detected in an equalized-feeding experiment on this scale.

The comparative utility of the four tests proposed here may be summarized as follows. Methods I and II are crude but have the advantage of being quickly applicable. Method I is conclusive if it gives a strongly significant result: method II, if it gives a non-significant result. The median test is somewhat more delicate but still fails to use all the data. The Q-test is the

most sensitive but is considerably more tedious to apply and involves a slight element of arbitrariness which is absent from the others.

REFERENCES

(1) Henry, Ikin & Kon (1937). *J. Dairy Res.* **8**, 282.
(2) White (1937). *Milk and Nutrition*, Part I, p. 64. Reading: Nat. Inst. Res. Dairying.
(3) Fisher, R. A. (1934). *Statistical Methods for Research Workers*, 5th ed. Edinburgh and London: Oliver and Boyd.

(*MS. received for publication* 5 *July* 1937)

636.2.084.1:612.392.84

166. THE RELATIVE VALUES OF RAW AND PASTEURIZED MILK IN THE FEEDING OF CALVES

By J. WILKIE, S. J. EDWARDS, A. B. FOWLER and N. C. WRIGHT

From the Hannah Dairy Research Institute, Kirkhill, Ayr

Previous work on the relative value of raw and pasteurized milk for calves has already been reviewed by Stirling & Blackwood (1) and by McCandlish & Black (2). Since the publication of these reviews a paper has been published by Wilson *et al.* (3) who were unable to find any significant differences in the growth rates of two groups of calves fed respectively on raw and pasteurized milk. Blackwood *et al.* (4), in a series of metabolism experiments, found no significant differences in the assimilation and retention of nitrogen, phosphorus and calcium. The milk used in both these experiments was, however, derived from a tuberculin-tested herd, and pasteurization was carried out on a laboratory scale. The investigation reported in the present paper was designed to determine the relative values of raw and pasteurized milk for calves *when produced and processed under typical commercial conditions.*

Method of experiment

Milk

Milk for the experiments was obtained from a local depot which was licensed to produce "Pasteurized" milk under the Milk (Special Designations) Order (Scotland) 1923. At this depot the milk of ten farms, amounting to some 400 gall., was bulked each morning in the receiving tank from which, after thorough mixing, raw milk was taken. Thereafter the remainder was run through the holder pasteurizing plant,[1] the processed milk being collected directly into churns under the cooler. In order to avoid any possibility of its being mixed with other milk in the plant, the necessary amount of pasteurized milk for the day's feed was taken from the first run of each morning. Holding temperature charts were kept each day.

Milk control

On arrival at the Institute two samples were taken from each can. Kay & Graham's phosphatase test was carried out on one set of samples every morning before feeding in order to check the efficiency of pasteurization.

[1] The plant was of the Pfaudler type. The milk was preheated to a temperature of 145° F. before passing into the batch pasteurizer (jacketed type) of 130 gall. capacity. The temperature of the milk in the holder was raised to and maintained at 147·5° F. for half an hour.

Daily butterfat tests were also carried out on these samples as a check on the quality and identity of the milk. The results of both tests were uniformly satisfactory. The second set of samples was set aside for guinea-pig inoculation, the gravity cream and sediment of combined daily aliquots of the milk (50 ml.) being inoculated twice a week into duplicate guinea-pigs, which were examined post-mortem at 8 weeks. As a further check on the efficiency of pasteurization blood was taken from the guinea-pigs at the time of death and tested for *Br. abortus* agglutinins. Weekly samples of both types of milk were taken for bacteriological examination.

Animals

Because of sex differences in response to feeding, and in view of the claim put forward by McCandlish & Black (2) that the nutritive value of pasteurized milk for bull calves is lower than for heifer calves, the former were used in the present experiments. The calves were obtained from tuberculin-tested Ayrshire herds, and were collected at 2–3 days of age, when they had received two to five feeds of colostrum. As collected the calves were tattooed in serial order, and, on arrival at the Institute, odd numbers were allocated to the pasteurized milk-fed group and even numbers to the raw milk-fed group. The allocation of calves into groups was therefore a random one.

Housing

This was arranged in two isolated units, each consisting of three huts. The units were over a quarter of a mile apart and were furnished at their entrances with foot-baths containing strong disinfectant solution.[1] No other stock had access to these huts. Each unit had its own attendant. The huts were 18×9 ft. in area and were built of $\frac{1}{2}$ in. weather boarding on 2×2 in. uprights. The roofs were covered with felt. Two huts in each group were used for housing the calves, these being fitted inside with wooden rails on which were mounted clips for pails. Each calf was tethered in its place by means of a heavily plated chain with a spring link at each end. Each hut accommodated ten calves but separate pens were not provided. Between feeding the calves therefore had ample opportunity of exercise. Sawdust was used as bedding throughout the tests. Provision was made for the storage, washing and sterilization of utensils in the third hut. A maximum-minimum thermometer was suspended in each stock hut and daily readings were taken at 9 a.m. The mean maximum values were 57 and 55° F. for the pasteurized and raw milk-fed groups respectively. The mean minimum value was 43° F. for both groups.

[1] In a preliminary trial with twenty-four calves each group was housed in three pens. These two sets of pens were adjacent and under the same roof. A single attendant was responsible for feeding both groups of calves. At the conclusion of the trial reactors to the tuberculin test were found in both groups. This trial showed the necessity for the rigid precautions and strict isolation maintained in the present study.

Feeding

The milk was mixed and heated to 100° F. before being fed. Each calf was fed 1 pt. of milk on the day of arrival at the Institute, and this amount was progressively increased until, at roughly the seventh day, the calf was receiving daily amounts equal to one-tenth of its body weight. Thereafter, each calf was weighed weekly and the daily amount of milk fed during any one week was at the rate of 1 kg. per 10 kg. body weight at the commencement of that week. The two groups were simultaneously fed twice daily. Individual numbered pails, fitted with teats, were used, and within each group the calves were fed in numerical order. In addition to milk at the above rate an average allowance of 2/3 lb. of hay per head per day was offered from the eighth to the twelfth week of life.

Recorded data

Post-mortem examinations were conducted on the carcasses of all calves which died or were slaughtered in the course of the experiment. Live weights were noted weekly. The following measurements for each calf were taken in the first and twelfth weeks of life: height, length, girth, and circumference of pastern bone. At the conclusion of the experiment calves were photographed individually against a measured background. They were also paraded before an independent panel of judges in order that their condition might be assessed. The calves were then subjected to the double intradermal test and were finally slaughtered, post-mortem examinations being made of the carcasses.

RESULTS

In the course of the present study two trials were carried out, one in 1935 and one in 1936. A preliminary trial with calves born in the spring of 1935 had already shown a death-rate of 62%, the numbers of deaths in the pasteurized milk-fed group being eighteen and in the raw milk-fed group twenty-one. Of these deaths 40% were attributable to *Bact. coli* septicaemia, 37% to lung infections, and the remainder to other miscellaneous pathological conditions. Accordingly, the two present trials were carried out with calves born in the autumn, since, according to Jordan (5), mortality among autumn calves tends to be lower than among spring calves.

Since the conditions of the two tests were identical, it was considered justifiable to combine the results for the two years. This view is confirmed by an examination of the weight increases of the calves which survived up to 12 weeks on the experimental feeding:

Year	Total no. of calves	Percentage increase in weight of pasteurized and raw milk-fed groups combined
1935	34	77·8
1936	39	77·0

Health and death-rate

In the course of the experiments ninety-two bull calves were secured. Of these, nineteen died before completion of 12 weeks on the milk ration. The distribution of ages at death of these calves is shown in Table I. It will be seen that, out of the nineteen deaths, five occurred in the pasteurized milk-fed group and fourteen in the raw milk-fed group, the average ages at death being 44 and 27 days respectively.

In respect of the calves in the pasteurized milk-fed group acute gastritis was found in two and pneumonia in one. In the raw milk-fed group acute gastritis was found in two and pneumonia in seven. No abnormal lesions were found in the remaining seven calves. Symptoms of white scour were not observed in any of the calves.

Table I. *Distribution of ages at death*

Age days	Milk group: Pasteurized (5 calves)	Milk group: Raw (14 calves)
5	—	1
8	—	1
11	—	1
15	—	1
16	1	1
17	1	—
19	—	1
23	—	1
27	—	1
32	—	2
36	—	1
39	1	—
40	—	1
45	—	1
59	1	—
86	—	1
91	1	—
Mean age at death (days)	44·4	27·1

Live-weight increases

Details of the live-weight increases of the seventy-three surviving calves are given in Table II, where the arithmetic means, standard deviations and standard errors are also shown. The distribution of the percentage increases in live weight of the two groups are recorded in Table III, the class intervals being 15% and the mean value for all calves 77·5%.

At first sight it might appear that the percentage increase in weight of the pasteurized milk-fed group was more satisfactory than that of the raw milk-fed group, the respective figures being $80{\cdot}2 \pm 3{\cdot}1$ and $74{\cdot}5 \pm 2{\cdot}7$. The mean percentage increase of the former group exceeded that of the latter group by almost 6%. The distributions of the two groups also show apparent differences in favour of the pasteurized milk-fed calves. An examination of the standard errors of the values within each group shows, however, that the observed

Table II. *Live-weight increases to* 12 *weeks of age.*

Pasteurized milk-fed group					Raw milk-fed group				
Calf no.	Weight (kg.) Initial	Final	Increase	% increase	Calf no.	Weight (kg.) Initial	Final	Increase	% increase
1	33·50	75·00	41·50	124	4	31·00	54·00	23·00	74
5	34·25	69·50	35·25	103	6	36·50	65·50	29·00	79
9	29·50	56·50	27·00	92	8	28·50	56·50	28·00	98
11	32·50	65·00	32·50	100	10	32·25	58·75	26·50	82
13	32·25	63·50	31·25	97	12	34·00	65·50	31·50	93
15	28·50	62·50	34·00	119	14	28·00	48·75	20·75	74
17	26·00	48·50	22·50	87	16	30·50	49·00	18·50	61
19	42·00	75·50	33·50	80	18	32·00	46·25	14·25	45
21	24·75	38·50	13·75	56	22	31·25	54·00	22·75	73
23	31·50	58·50	27·00	86	26	30·75	48·00	17·25	56
25	29·75	56·25	26·50	89	28	29·75	48·50	18·75	63
29	33·00	57·00	24·00	73	30	36·00	65·00	29·00	81
31	31·50	46·50	15·00	48	34	29·75	54·00	24·25	82
35	38·50	68·50	30·00	78	36	35·50	61·00	25·50	72
37	32·50	64·00	31·50	97	38	27·75	53·00	25·25	91
41	34·25	52·50	18·25	53	40	36·50	51·50	15·00	41
43	29·00	41·00	12·00	41	44	34·25	55·50	21·25	62
A 1	38·75	72·50	33·75	87	A 2	33·00	65·75	32·75	99
A 3	35·00	61·25	26·25	75	A 4	30·25	55·75	25·50	84
A 5	31·25	57·75	26·50	85	A 6	29·75	61·50	31·75	107
A 7	30·75	44·25	13·50	44	A 8	29·25	49·25	20·00	68
A 9	30·00	57·75	27·75	92	A 10	29·50	57·00	27·50	93
A 11	34·00	64·75	30·75	90	A 12	33·50	59·50	26·00	78
A 13	33·50	63·75	30·25	90	A 14	36·25	62·00	25·75	71
A 15	29·50	55·75	26·25	89	A 18	31·75	55·75	24·00	76
A 17	29·50	53·50	24·00	81	A 20	28·25	53·50	25·25	89
A 19	28·25	56·75	28·50	101	A 22	25·75	37·25	11·50	45
A 21	26·75	49·75	23·00	86	A 24	27·00	53·75	26·75	99
A 23	32·00	50·75	18·75	59	A 26	26·25	43·00	16·75	64
A 25	32·25	56·25	24·00	74	A 28	29·25	53·75	24·50	84
A 27	35·50	56·25	20·75	58	A 30	25·75	41·50	15·75	61
A 29	33·75	60·75	27·00	80	A 32	32·75	56·50	23·75	72
A 31	33·25	59·75	26·50	80	A 34	30·00	47·75	17·75	59
A 33	26·25	48·00	21·75	83	A 36	32·00	53·00	21·00	66
A 35	33·00	55·25	22·25	67	A 38	33·75	63·00	29·25	87
A 37	34·50	54·25	19·75	57	A 40	28·75	43·75	15·00	52
A 39	37·25	62·75	25·50	68					
Mean	32·11	57·85	25·74	80·24	Mean	31·03	54·11	23·08	74·47

Mean percentage increase = 80·2 ± 3·1.* Standard deviation = 19·10.

Mean percentage increase = 74·5 ± 2·7.* Standard deviation = 16·00.

Difference in percentage live weight increase between the two groups = 5·7 ± 4·1.*

* Standard errors used throughout the study.

Table III. *Distribution of percentage increases in live weight to* 12 *weeks of age*

Percentage increase in live weight	Pasteurized milk-fed calves	Raw milk-fed calves
41– 55	4	4
56– 70	6	9
71– 85	10	14
86–100	13	8
101–115	2	1
116–130	2	0
Total calves	37	36

differences are not significant. The standard deviations are, moreover, very large, and serve to demonstrate the great difficulty which is entailed in securing calves which will give similar growth rates even when fed on the same type of milk. In the past this fact appears to have been frequently overlooked, and workers have tended to base their conclusions upon entirely inadequate numbers of calves. Examination of Table III shows the wide "spread" in the percentage live-weight increases. The values range from 41 to 124% for the pasteurized milk-fed group and from 41 to 107% for the raw milk-fed group. Similar differences between individuals within the same group have been recorded by other workers. McCandlish & Black (6) record extreme values of 28 and 175% for a group of pasteurized milk-fed calves and of 45 and 245% for a group of raw milk-fed calves. Results obtained by Wilson *et al.* (3) show 38 and 82% for the former and 47 and 76% for the latter.[1]

The weekly percentage increases in weight of the two groups are given in Table IV. These figures show that the mean growth rate was regular and that the growth of the calves on the pasteurized milk ran strictly parallel with those on the raw milk.

Table IV. *Growth rates. Group weights at end of each week expressed as a percentage of initial group weight*

Season		Weeks on test: 1	2	3	4	5	6	7	8	9	10	11	12
Pasteurized milk-fed group													
1935	100	105	107	112	115	121	130	138	145	153	163	171	184
1936	100	104	111	112	116	121	126	132	139	148	157	166	177
Raw milk-fed group													
1935	100	100	105	109	113	118	122	130	136	145	154	164	172
1936	100	106	110	114	117	122	129	132	140	150	162	166	177

It will be shown later that a number of the raw milk-fed calves were infected with tuberculosis, and it might be argued that the presence of this disease would interfere with the growth of the calves and so invalidate comparison between the raw and pasteurized milk-fed groups. On examination, it was found that the twenty-four reacting calves in the raw milk-fed group gave a percentage live-weight increase of $73{\cdot}0 \pm 3{\cdot}4$, while the twelve non-reacting calves in the same group gave a value of $77{\cdot}4 \pm 4{\cdot}2$. The difference, i.e. $4{\cdot}5 \pm 5{\cdot}3$, is not significant. At this stage of life, therefore, the presence of tuberculosis did not apparently interfere with the growth rate.

It may be remarked that the live-weight increases per day were much smaller than those generally reported in the literature.

This may be partly due to the short period on colostrum, to the relatively low rate of feeding at the commencement of the experiment, to the low temperature of housing, and to the entire freedom of movement afforded to the calves in the huts. It also appears to be partly due to the lack of any supplementary feeding in the present tests. In order to illustrate this point the percentage increases in the weight of the calves to 8 weeks of age are compared

[1] These figures refer to growth at 8 weeks.

in Table V with the results obtained by McCandlish & Black (2, 6) and by Wilson *et al.* (3). In McCandlish & Black's experiments the calves received hay

Table V. *Percentage live-weight increases of calves to* 8 *weeks*

Investigation	Pasteurized milk-fed group	Raw milk-fed group
McCandlish & Black (2)	92·6	97·8
McCandlish & Black (6)	77·6	76·2
Wilson *et al.* (3)	62·9	61·2
Present investigation	43·3	38·4

and concentrates in addition to milk, while Wilson *et al.* fed a supplement of hay only. In the present experiment milk was fed to 8 weeks of age without any supplement. It will be seen that the growth rates with the hay and concentrate supplement were as much as twice that when milk alone was fed, although the two former series of experiments show unexplained differences. The growth rate of the calves receiving the hay supplement was intermediate. In each of the above tests milk was fed at the rate of 1 lb. daily per 10 lb. body weight, though at a later stage in their experiment Wilson *et al.* fed up to 20 oz. of milk per 10 lb. body weight per day to certain calves. Growth rate thus appears to increase with an improvement in the nutritive value of the supplementary ration, a fact which is commonly recognized by practical breeders. In the present experiments supplementary feeding was deliberately omitted during the first 8 weeks, in order that any minor differences in the nutritive value of the two classes of milk might not be masked. Even if the growth rate was relatively low, the results for the two groups are strictly comparable.

Conformation

The measurements of the calves at the commencement and at the end of the experiment are given in Table VI. It will be seen that there were no appreciable differences between the two groups.

Table VI. *Conformation*

	Height in.	Girth in.	Length in.	Pastern in.
	Pasteurized milk-fed group			
Initial mean	27·53	28·66	27·15	4·15
Standard deviation	1·22	1·23	1·52	0·92
Final mean	32·01	35·51	31·69	4·48
Standard deviation	1·59	2·42	1·92	1·83
	Raw milk-fed group			
Initial mean	27·40	28·46	26·69	4·13
Standard deviation	1·22	1·19	1·58	0·17
Final mean	31·35	33·88	31·33	4·37
Standard deviation	1·54	2·82	2·21	0·14

Condition of calves at end of experiments

At the conclusion of the experiments the calves were examined as to general health and development by a panel of four independent judges. Three were well-known judges of Ayrshire stock; the fourth was a veterinary surgeon

with wide experience of the breed. They were asked to place the calves in three classes; class A being those in exceptionally good condition, class B being those in a satisfactory condition, and class C those in a definitely unthrifty condition. The judges were of course unaware of the nature of the milk fed. Their awards are given in Table VII. It will be seen that these showed con-

Table VII. *Judges' reports upon live calves on a points basis.*

Calf in Class A: 3 points. Calf in Class B: 2 points. Calf in Class C: 1 point

Pasteurized milk-fed group					Raw milk-fed group				
	Judges' awards					Judges' awards			
Calf no.	P	Q	R	S	Calf no.	P	Q	R	S
1	3	2	3	3	4	3	2	2	2
5	3	3	2	3	6	3	2	2	3
9	3	2	1	2	8	3	3	3	3
11	3	2	1	3	10	2	2	2	2
13	3	2	1	1	12	2	3	3	3
15	3	2	2	2	14	2	1	2	3
17	3	1	2	2	16	1	2	1	1
19	3	3	3	3	18	1	1	1	2
21	2	1	1	1	22	1	1	1	1
23	3	2	2	2	26	2	1	1	2
25	3	2	1	2	28	1	1	1	1
29	3	2	3	3	30	3	2	2	3
31	2	1	2	2	34	2	2	2	2
35	3	2	3	3	36	3	1	3	3
37	3	2	2	3	38	2	1	1	2
41	3	2	2	2	40	1	1	1	1
43	1	1	1	1	44	2	2	3	3
A 1	3	3	3	3	A 2	3	3	3	3
A 3	3	2	2	3	A 4	3	3	3	3
A 5	3	2	2	2	A 6	3	2	3	3
A 7	1	1	1	2	A 8	2	2	2	2
A 9	2	3	3	3	A 10	2	2	3	3
A 11	2	2	2	2	A 12	3	2	3	3
A 13	3	3	3	3	A 14	3	3	3	3
A 15	3	2	2	2	A 18	2	2	1	2
A 17	3	2	3	2	A 20	2	2	2	2
A 19	3	3	3	3	A 22	1	2	1	1
A 21	3	2	2	2	A 24	2	2	2	2
A 23	2	2	2	2	A 26	2	1	1	1
A 25	2	2	3	3	A 28	2	2	2	2
A 27	3	2	2	3	A 30	1	1	1	1
A 29	3	3	3	3	A 32	2	3	3	3
A 31	3	2	3	3	A 34	3	2	2	1
A 33	2	2	1	2	A 36	2	2	1	1
A 35	3	2	3	2	A 38	3	3	3	3
A 37	3	2	2	2	A 40	2	2	1	2
A 39	3	2	3	3					
Total for group	100	76	80	88	Total for group	77	69	71	78

Analysis of Judges' awards

Pasteurized milk-fed group					Raw milk-fed group				
	Judges' awards					Judges' awards			
Class	P	Q	R	S	Class	P	Q	R	S
A	28	7	14	17	A	12	7	12	15
B	7	25	15	17	B	17	19	11	12
C	2	5	8	3	C	7	10	13	9

sistent differences in favour of the pasteurized milk-fed group, though the significance of such differences cannot be assessed. It may be noted that the greatest difference between the two groups was recorded by the veterinary surgeon (designated "P"), who might be expected to pay special attention to the health of the animals.

Tuberculin tests and post-mortem results

It has already been mentioned (p. 312) that grouped daily aliquot portions of all the milk fed were inoculated twice weekly into guinea-pigs to determine the presence or absence of viable tubercle bacilli. The results of these inoculations are shown in Table VIII. It will be seen that, out of the forty-seven raw-

Table VIII. *Incidence of* Myc. tuberculosis *and* Br. abortus *in grouped aliquot samples of milk*

Year	Total samples	*Myc. tuberculosis* Number infected	*Myc. tuberculosis* Percentage infected	*Br. abortus* Number infected	*Br. abortus* Percentage infected
		Pasteurized milk-fed group			
1935	21	0	0	0	0
1936	24	0	0	0	0
Total	45*	0	0	0	0
Additional daily samples	12†	0	0	0	0
		Raw milk-fed group			
1935	23	16	70	7	30
1936	24	17	71	11	46
Total	47*	33	70	18	38
Additional daily samples	12†	8	67	5	42

* The total number of samples examined was sixty-four pasteurized and sixty-four raw. In nineteen tests of pasteurized and in seventeen tests of raw milk, guinea-pigs died prematurely.

† In addition to the grouped aliquot samples, a series of twelve samples of each type of milk was examined daily at the conclusion of the second trial, in order to investigate the day to day incidence of infection.

milk samples in which inoculated guinea-pigs survived the experiment, thirty-three showed the presence of viable tubercle bacilli. On the other hand, an examination of forty-five samples of the pasteurized milk failed to reveal a single positive result. *Br. abortus* was found in eighteen samples of raw milk, but was absent from the pasteurized samples.

In so far as tuberculosis is concerned, these differences were reflected in the results of the tuberculin tests and the post-mortem examinations which were carried out on the calves at the conclusion of the experiment. The results are shown in Table IX. It will be seen that twenty-four out of the thirty-six calves fed on raw milk reacted to the test. With a single exception post-mortem examinations showed the presence of active tuberculous lesions in the reacting calves. Among the pasteurized milk-fed calves only one gave a positive

Table IX. *Results of tuberculin tests and post-mortem examinations*

Pasteurized milk-fed group					Raw milk-fed group				
	Tuberculin tests					Tuberculin tests			
Calf no.	First reading 48 hr. mm.	Second reading 72 hr. mm.	Reaction	Post-mortem findings at abattoir	Calf no.	First reading 48 hr. mm.	Second reading 72 hr. mm.	Reaction	Post-mortem findings at abattoir
1	7	10	–		4	6	8	–	
5	7	8	–		6	6	7	–	
9	7	8	–		8	7	8	–	
11	7	8	–		10	26	34	+	T.B. bronchial glands
13	6	7	–		12	24	30	+	T.B. bronchial glands. Slight T.B. of pleura and small caseous area in anterior lobe of left lung
15	7	9	–		14	7	8	–	
17	6	7	–		16	30	32	+	T.B. bronchial glands and anterior lobe of left lung. T.B. hepatic glands
19	6	6	–		18	6	7	–	
21	5	5	–		22	5	5	–	
23	5	6	–		26	18	22	+	T.B. bronchial glands
25	32	37	+	No tuberculous lesions found and negative results from guinea-pig inoculations of glandular material	28	50	55	+	T.B. bronchial glands and anterior lobes of both lungs
29	6	7	–		30	7	8	–	
31	7	7	–		34	6	9	–	
35	7	10	–		36	7	10	–	
37	6	7	–		38	6	7	–	
43	6	7	–		40	25	28	+	T.B. bronchial glands and anterior lobe of right lung
41	8	8	–	Suppurating foci in lungs, non-tuberculous in origin	44	7	9	–	

Table IX (*continued*)

A 1	6	6	–		A 2	5	6	–	
A 3	5	5	–	Abscess in liver	A 4	10	16	+	T.B. bronchial glands. Also anterior and middle lobes of both lungs
A 5	5	6	–		A 6	22	19	+	T.B. bronchial and mediastinal glands
A 7	5	5	–	Abscess in liver	A 8	18	19	+	T.B. bronchial glands
A 9	5	6	–		A 10	24	30	+	No lesions revealed in careful naked-eye search
A 11	5	5	–		A 12	17	18	+	T.B. bronchial and mediastinal glands, also anterior middle lobes of both lungs
A 13	6	6	–		A 14	20	24	+	T.B. bronchial glands
A 15	5	6	–		A 18	24	28	+	T.B. bronchial and mediastinal. Slight pleurisy
A 17	4	6	–		A 20	12	18	+	T.B. bronchial and mediastinal glands and lung
A 19	7	7	–		A 22	16	14	+	T.B. bronchial and mediastinal glands
A 21	7	8	–		A 24	10	20	+	T.B. bronchial and mediastinal glands and lung
A 23	5	6	–		A 26	20	22	+	T.B. bronchial glands and lung
A 25	6	6	–		A 28	22	20	+	T.B. bronchial glands and lung
A 27	5	6	–		A 30	18	22	+	T.B. bronchial glands and lung
A 29	5	6	–		A 32	21	26	+	T.B. bronchial glands and lung
A 31	5	6	–		A 34	22	26	+	T.B. bronchial glands and lung
A 33	4	5	–		A 36	22	24	+	T.B. bronchial glands and lung
A 35	5	6	–		A 38	15	16	+	T.B. bronchial and mediastinal glands and lung
A 37	5	6	–		A 40	22	20	+	T.B. bronchial and mediastinal glands and lung
A 39	5	6	–						

reaction to the tuberculin test. Moreover, exhaustive post-mortem examination failed to reveal any tuberculous lesions in this calf, and inoculation of glandular material into four guinea-pigs failed to reveal the presence of tubercle bacilli. It is apparent from these facts that the positive reaction in this calf must be considered an anomalous one. Such anomalous reactions have occasionally been recorded in the literature (Jordan (7); McEwen & Roberts (8)).

The nature of the tuberculous lesions in the affected calves may be noted. Infection was most frequently found in the bronchial glands, whereas no macroscopic lesions were found in the mesenteric glands. This fact would ordinarily be held to indicate that infection took place by inhalation. However, since one calf in each hut must have contracted infection from the milk by ingestion, it is not unreasonable to suppose that all the other calves became infected in the same manner and that transmission from one calf to the other in the same hut did not account for the high incidence of tuberculosis in the calves fed on raw milk. In support of this view it may be stated that many of the lesions were in an advanced stage, calcification being present. This indicates that infection must have taken place at an early period in the experiment as a result of the very massive infection present in the raw milk. In connexion with the above findings it is of interest to quote the views of Gofton (1937): "Although I cannot prove it, I strongly suspect that quite a number of the cases in which there are primary lesions confined to the bronchial or mediastinal glands, have their origin in alimentary infection."

Summary and conclusions

1. Bull calves from tuberculin-tested Ayrshire herds were fed on raw or commercially pasteurized milk up to 12 weeks of age, in amounts strictly in relation to their body weight. This diet was supplemented from the eighth week by hay at the rate of 2/3 lb. per head per day.

The milk used was mixed milk from untested herds, the raw and pasteurized milk being derived from the same bulk sample. The quality of the milk was controlled by daily butterfat and phosphatase tests and by inoculation of grouped daily aliquots twice weekly into duplicate guinea-pigs.

2. The total number of calves used in the experiment was ninety-two. Of these, forty-two received pasteurized milk and fifty raw milk. Nineteen calves died before completion of the experiment, five in the pasteurized milk-fed group and fourteen in the raw milk-fed group. The mean ages of these calves at death were 44 and 27 days respectively.

Seventy-three calves completed the full 12 weeks of the experimental feeding. Of these, thirty-seven received pasteurized milk and thirty-six raw milk. The former group showed a mean percentage gain in live weight of $80{\cdot}2 \pm 3{\cdot}1$, the corresponding figure for the latter group being $74{\cdot}5 \pm 2{\cdot}7$. The difference, i.e. $5{\cdot}7 \pm 4{\cdot}1$, was not significant. Reasons are given for the relatively low growth rate observed.

3. No appreciable differences were noted in the skeletal growth of the two groups. Marks awarded by experienced stock judges showed consistent differences in favour of the pasteurized milk-fed group, although the significance of such differences cannot be assessed.

4. Inoculations of grouped daily aliquots of raw milk twice weekly into duplicate guinea-pigs resulted in finding viable tubercle bacilli in 70% of the samples and *Br. abortus* in 38% of them. The pasteurized milk samples were uniformly negative to both tests.

The differences in tuberculous infection of the two types of milk were reflected in the results of tuberculin tests and post-mortem examinations on the calves at the conclusion of the experiment. Twenty-four out of thirty-six calves fed on raw milk reacted to the test, and the presence of tuberculous lesions was confirmed in twenty-three by post-mortem examination. One calf in the pasteurized milk-fed group reacted to the test, but exhaustive post-mortem examination and inoculation of glandular material into guinea-pigs failed to confirm the presence of any tuberculosis.

5. This work has failed to show any significant differences in the nutritive value of raw and of pasteurized milk for the rearing of young calves. The use of pasteurized milk, however, had a clear advantage in that it preserved the animals from infection through drinking milk containing living tubercle bacilli.

ACKNOWLEDGEMENTS

We are indebted to United Dairies, Ltd., for a grant in aid of the expenses of this investigation, and to the Scottish Co-operative Wholesale Society, Ltd., for undertaking the collection and processing of the milk. We are also most grateful to Dr F. C. Minett for his interest and help throughout the work, and for his assistance at the post-mortem examinations. Miss A. A. Nichols kindly undertook the necessary bacteriological tests.

REFERENCES

(1) STIRLING & BLACKWOOD (1933). *Bull. Hannah Dairy Inst.* No. 5.
(2) MCCANDLISH & BLACK (1935). *Res. Bull. W. Scot. agric. Coll.* No. 4.
(3) WILSON, MINETT & CARLING (1937). *J. Hyg., Camb.*, **37**, 243.
(4) BLACKWOOD, MORRIS & WRIGHT (1936). *J. Dairy Res.* **7**, 228.
(5) JORDAN (1933). *Vet. J.* **89**, 202.
(6) MCCANDLISH & BLACK (1932). *Scot. Fmr*, **40**, 1682, 1713 and 1744.
(7) JORDAN (1933). *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 184.
(8) MCEWEN & ROBERTS (1934). *J. comp. Path.* **47**, 32.

(*MS. received for publication* 25 *June* 1937)

637.148

167. DISCOLORATION AND CORROSION IN CANNED CREAM. PART II

By C. J. JACKSON, G. R. HOWAT and T. P. HOAR

From The Hannah Dairy Research Institute, Kirkhill, Ayr, and The Metallurgical Laboratories, University of Cambridge

A preliminary investigation of certain defects and the results of the short-time storage of experimentally processed batches of cream have been reported in a previous communication (1). The results of further storage of these batches and of additional batches utilizing different stabilizing agents are now presented. It is thought that a complete picture of the corrosion phenomena and the main factors influencing them have now been obtained.

Influence of long-time storage on batches previously reported

Tables I–III record observations on cans from several batches after 7 months' storage, with some of the previously published results included for comparison. In each of these and all other tables, – indicates no effect, + slight effect, + + considerable effect, + + + severe effect, etc.

Table I. *Effect of sterilization time*

	Stabilizer (g. $NaHCO_3$ per gall.)	*p*H before sterilization	Sterilization		2 weeks			7 months			
Batch			Temp. °C.	Time min.	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	Condition of cream
1	5·0	6·89	117–118	25	6·45	–	+	6·39	–	+	—
	5·0	6·89	117–118	30	6·48	–	–	6·35	–	+	—
	5·0	6·89	117–118	40	6·34	+	–	6·35	+	+	—
	5·0	6·89	117–118	50	6·48	+ +	–	6·40	+ + +	+	—

The main effect of storage is seen to be an all-round increase of pitting.

Table II. *Effect of sterilization temperature*

	Stabilizer (g. $NaHCO_3$ per gall.)	*p*H before sterilization	Sterilization		2 weeks			7 months			
Batch			Temp. °C.	Time min.	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	Condition of cream
2	5·0	6·83	114–115	25	6·69	–	–	6·61	–	+	—
	5·0	6·83	114–115	40	6·45	–	+	6·36	–	+	—
	5·0	6·83	117–118	25	6·40	–	–	6·36	–	+	—
	5·0	6·83	117–118	40	6·38	+	–	6·29	+ +	+	—
3	5·0	6·91	114–115	25	6·47	–	–	6·43	–	–	—
	5·0	6·91	114–115	40	6·40	–	–	6·35	–	+	—
	5·0	6·91	117–118	25	6·50	–	–	6·45	–	–	—
	5·0	6·91	117–118	40	6·56	+ +	–	6·45	+ +	–	—

Increased pitting with storage is again evident; the cans which showed purpling after 2 weeks show reduced purpling but greatly increased pitting after

7 months. Evidently the higher temperature and longer time of sterilization leads eventually to increased pitting as well as to increased purpling in the early stages.

Table III. *Effect of stabilizer*

Batch	Stabilizer (g. $NaHCO_3$ per gall.)	*p*H before sterilization	Sterilization		2 weeks			7 months			
			Temp. ° C.	Time min.	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	Condition of cream
4	None	6·56	117–118	40	6·30	+	–	6·28	+	–	—
	1·0	6·63	117–118	40	6·28	–	–	6·15	–	+ +	—
	3·0	6·71	117–118	40	6·26	–	–	6·18	–	+	—
	5·0	6·88	117–118	40	6·30	–	+	6·25	–	+	—
5	3·0	6·81	117–118	25	6·51	–	–	6·45	–	–	—
	4·0	6·84	117–118	25	6·46	–	–	6·42	–	+	—
6	None	6·36	117–118	25	6·43	+	–	6·26	–	+	—
	None	6·36	117–118	40	6·07	–	–	6·01	+	+ +	—
	5·0	6·78	117–118	25	6·50	–	–	6·46	–	+	—
	5·0	6·78	117–118	40	6·34	–	–	6·28	+ +	–	—
7	None	6·54	114–115	25	6·39	–	–	6·29	–	+	—
	None	6·54	114–115	40	6·19	–	–	6·27	–	–	—
	5·0	6·91	114–115	25	6·52	–	–	6·47	–	–	—
	5·0	6·91	114–115	40	6·35	+ ?	–	6·29	–	+	—
8	None	6·50	114–115	25	6·35	–	–	6·27	–	+	Black film and spots, cont. Fe
	None	6·50	114–115	40	6·18	–	–	6·05	–	+	Slight black film
	5·0	6·95	114–115	25	6·55	–	–	6·45	–	+	Black film and spots, cont. Fe and Sn
	5·0	6·95	114–115	40	6·40	–	–	6·35	–	+	—

Pitting is again more pronounced; in two cases cans which showed purpling after 2 weeks show only pitting after 7 months. Sodium bicarbonate up to 5 g. is seen to have as little influence on the progress of pitting as on the purpling previously reported.

Further experimental batches

In the following further experiments, the processing procedure was similar in all respects to that adopted in the first batches and detailed in the previous paper, except that the homogenization pressure was kept constant at 3300 lb./in.^{2} and the sterilization temperature at 117–118° C. The analysis of most of the creams used in these batches is given in Table IV.

Table IV. *Analysis of experimental creams, second series*

Batch	Butterfat %	Titratable acidity (ml. *N*/10 NaOH)	*p*H (quinhydrone)	N mg./100 g.		
				Total	Non-casein	Non-protein
13			Not determined			
14	23	1·4	6·83	428	131	32
15	23	1·3	6·93	407	128	23
16	23	1·4	6·88	415	131	34
17			Not determined			
18	23	1·2	6·83	436	124	31
19	22	1·3	6·77	410	125	35
20	25	1·0	6·90	386	116	23
21	25	1·0	6·95	387	144	30
22	25	1·3	6·76	396	110	25
23	23	1·0	6·83	349	98	21
24	25	1·3	6·89	352	116	29

Influence of excessive amounts of sodium bicarbonate

Since it was observed, as previously reported, that the additions of sodium bicarbonate to cream assists the production of sulphydryls on heating, it was thought desirable to ascertain the effects produced by the addition of amounts larger than 5 g. per gallon to cream processed in cans in the normal way. Table V records observations of cans containing the larger amounts of added bicarbonate.

It is clear that large amounts of bicarbonate favour the production of purpling in the early stages. Furthermore, the purpled cans become the most severely pitted in the later stages of storage and produce discoloration of the cream. The transition from purpling to pitting during storage is particularly clearly shown by the third condition in batch 13.

Influence of different stabilizers

Hunziker(2) has compared the relative merits of sodium bicarbonate, disodium monohydrogen phosphate and trisodium citrate as stabilizers from the point of view of the heat coagulation of milk, finding that phosphate and citrate offer certain advantages over bicarbonate. It therefore seemed of interest to compare the three salts as stabilizers for canned cream, from the point of view of corrosion and discoloration of the product. Table VI records observations of cans in a number of batches showing this comparison. Stabilizers were added in equimolecular amounts. This gave sufficient phosphate or citrate to bring the *p*H of the cream before sterilization to approximately the same value as that of the cream stabilized with bicarbonate.

It is clear that both phosphate and citrate in the amounts used are more liable to promote both purpling and pitting than is bicarbonate. In particular, if an average is taken of all the cans for each stabilizer after 7 months, it is seen that the mean degree of pitting produced in the bicarbonate samples is that designated +, while in the phosphate and citrate samples it is very nearly ++. Numerous cases where initial purpling has become transformed on storage into severe pitting will be noticed throughout the table. In general, the greatest amount of cream discoloration occurs in the most severely pitted cans, being more notable in the phosphate and citrate samples than in the bicarbonate, and favoured by increasing amounts of stabilizer. Some of the cans in these batches which had shown early purpling, showed after 7 months' storage small patches of a bronze or purple film on such parts of the tin as had been in the gas space, and thus out of contact with the liquid phase. From the general appearance of this stain it is thought to be a secondary film, formed after the initial purple film had disintegrated and been replaced by pitting.

It was noticed that in all batches there was a fall in *p*H of the cream after sterilization, and that this fall continued with storage. While it might be expected that the more acid creams would take up metal more readily, it is apparent from the results of the examination of commercially and experi-

mentally canned cream reported in this and the previous paper that there is no evident correlation between the acidity of the cream and the amount of metals taken up or corrosion produced.

It should be re-emphasized that owing to variations of cream from batch to batch, no completely accurate comparisons can be drawn as between batches. However, the considerable and consistent differences between batches as noted above are in all probability due to the deliberate variations of amounts of stabilizer, etc., rather than to chance variations of the creams.

DISCUSSION

(*a*) *Mechanism of the attack*

In a consideration of the mechanism of the attack found in this work, the following experimental facts may first be assembled. Purpling of the can, always associated with spangling, may appear immediately after processing or may develop within a period of a few weeks; after some months of storage it may diminish or disappear. Pitting is found only after a few weeks of storage, and is generally not marked until months have elapsed. It may occur in cans which show no evidence of purpling, but is severest in cans which have previously shown strong purpling, as evidenced by their etched appearance and the purpling actually found in similar cans opened earlier; for while strong purpling appears to inhibit pitting somewhat in the early stages of storage, it generally encourages it in the later. Black particles containing tin and iron, discolouring the cream, appear only in pitted cans, though a slight purple discoloration may sometimes occur on cream in strongly purpled cans which have not yet started to pit. A third type of attack, the bronze and purple "patchy" stain, appears only after long storage on such parts of a previously purpled can as have lacked constant contact with the liquid phase, when the parts in contact have lost the original purpling and appear etched and pitted.

These phenomena are probably caused as follows. The heat treatment of the cream, if too drastic, may produce sufficient sulphur compounds to cause attack of the tin, forming a coating of tin sulphide which adheres to the etched or spangled metal beneath. This effect is assisted by excessive amounts of stabilizing salts. On long storage, cans which have *not* been so attacked suffer slight localized pitting on the tin such as is often found on the metal when it is exposed to nearly neutral solutions, and which is considered by one of us elsewhere (3). On the other hand, cans which have been purpled with the formation of the sulphide film may undergo further change. The sulphide film, although comparatively thick and therefore protective in the early stages, gradually becomes undermined by the slow attack of the cream salts and acids, when the conditions are favourable. It may be that they actually adhere to a cream surface rather than to the metal, giving discoloration. The underlying metal is now considerably pitted by further attack, and steel may soon be exposed in some quantity, particularly as the removal of the fairly thick sulphide film has now resulted in a general loss of tin thickness.

Table V. *The effect of the addition of large amounts of bicarbonate*

Batch	Stabilizer (g. $NaHCO_3$ per gall.)	*p*H before sterilization	Sterilization time min.	20 hours			2 weeks			2 months			4 months			
				*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	Condition of cream
13	None	6·75	25	6·49	–	–	6·38	–	–	6·29	–	–	6·19	–	+	—
	5	7·03	25	6·84	–	–	6·76	–	–	6·51	–	–	6·52	–	+	—
	10	7·22	25	6·92	+ +	–	6·84	+ +	–	6·62	+	+ +	6·68	–	+ +	Black spots and film
	20	7·45	25	7·08	+ + +	–	6·99	+ + +	–	6·81	+ + +	–	6·81	+ + +	+ + +	Purple film
14	5	6·93	25	6·75	+	–	6·64	+	–	6·54	+	–	6·59	+	+	—
	15	8·00	25	7·26	+ + +	–	6·95	+ + +	–	6·92	+ + +	+	6·91	+ + +	+ +	—

Table VI. *The comparative effect of bicarbonate, phosphate and citrate*

Batch	Stabilizer	g. stabilizer per gall.	*p*H before sterilization	Sterilization time min.	20 hours			4 weeks			4 months			7 months*			
					*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	*p*H	Purpling	Pitting	Condition of cream
15	$NaHCO_3$	1·0	6·90	25	6·64	–	–	6·60	–	–	6·49	–	–	6·42	–	+	—
	$NaHCO_3$	1·0	6·90	40	6·61	–	–	6·66	+	–	6·51	+ +	–	6·43	+	+ +	—
	Na_2HPO_4	1·7	6·83	25	6·57	–	–	6·53	+	–	6·39	+	–	6·23	+ +	+	—
	Na_2HPO_4	1·7	6·83	40	6·57	–	–	6·48	+	–	6·33	–	+	6·29	–	+ + +	Black film
16	$NaHCO_3$	3·0	7·04	25	6·78	–	–	6·59	–	–	6·62	–	–	6·42	+	–	—
	$NaHCO_3$	3·0	7·04	40	6·79	+	–	6·55	+ +	–	6·60	+ +	–	6·47	+	–	—
	Na_2HPO_4	4·9	6·92	25	6·74	+	–	6·63	+ +	–	6·63	+	+	6·36	–	+	—
	Na_2HPO_4	4·9	6·92	40	6·65	+	–	6·55	+ +	–	6·54	+ +	–	6·38	+ +	+	—
17	$NaHCO_3$	5·0	7·05	25	6·88	–	–	6·73	–	–	6·66	–	–	6·57	+	–	—
	$NaHCO_3$	5·0	7·05	40	6·74	+ +	–	6·66	+ +	–	6·52	+ +	+	6·34	+	+ +	—
	Na_2HPO_4	8·15	6·96	25	6·63	+ + +	–	6·61	+ + +	–	6·56	+	+	6·49	+	+	—
	Na_2HPO_4	8·15	6·96	40	6·52	+ + + +	–	6·48	+ + + +	–	6·48	+ + +	+ +	6·35	+	+ +	—

Table VI (*continued*)

18	$NaHCO_3$	1·0	6·87	25	6·63	–	–	6·54	+	–	6·42	+	–	6·36	+	+	—
	$NaHCO_3$	1·0	6·87	40	6·58	–	–	6·44	–	–	6·30	+	–	6·29	+	+	—
	$Na_3C_6H_5O_7$	3·07	6·83	25	6·70	–	–	6·61	+	–	6·52	–	+	6·47	–	++	—
	$Na_3C_6H_5O_7$	3·07	6·83	40	6·58	–	–	6·54	–	–	6·49	–	+	6·35	+	+	—
19	$NaHCO_3$	3·0	7·23	25	6·84	–	–	6·89	–	–	6·76	–	–	6·74	–	–	—
	$NaHCO_3$	3·0	7·23	40	6·89	+	–	6·78	+	–	6·74	+	–	6·63	++	+	Black spots
	$Na_3C_6H_5O_7$	9·2	7·17	25	6·83	+	–	6·80	+	–	6·80	–	+	6·68	++	+	—
	$Na_3C_6H_5O_7$	9·2	7·17	40	6·73	+	–	6·88	++	+	6·68	++	++	6·57	++	+	—
20	$NaHCO_3$	5·0	7·12	25	6·89	–	–	6·75	–	–	6·67	–	–	6·61	–	–	—
	$NaHCO_3$	5·0	7·12	40	6·81	++	–	6·64	+++	–	6·58	–	++	6·42	–	+	—
	$Na_3C_6H_5O_7$	15·4	7·0	25	6·76	+	–	6·54	+++	–	6·52	++	+	6·34	+	++	Black spots
	$Na_3C_6H_5O_7$	15·4	7·0	40	6·63	++++	–	6·58	++++	–	6·50	++	+++	6·33	++	++++	Black spots
21	$NaHCO_3$	3·0	7·11	25	7·00	–	–	6·78	–	–	6·71	+	–	6·56	++	–	—
	Na_2HPO_4	4·9	6·95	25	6·72	–	–	6·56	–	–	6·51	+	–	6·42	+	+	—
	$Na_3C_6H_5O_7$	9·2	6·95	25	6·76	+	–	6·61	–	–	6·58	–	+	6·50	–	+	—
22	$NaHCO_3$	5·0	7·13	25	6·77	–	–	6·70	–	–	6·66	+	+	6·57	+++	+	—
	Na_2HPO_4	8·15	7·03	25	6·70	++	–	6·60	++	–	6·60	++	–	6·49	–	++	—
	$Na_3C_6H_5O_7$	15·4	7·00	25	6·75	++	–	6·59	++	–	6·49	++	–	6·53	–	+++	—
23	$NaHCO_3$	5·0	7·14	40	6·82	+++	–	6·73	+++	–	6·82	+++	–	6·61	++	++	—
	Na_2HPO_4	8·15	6·97	40	6·48	+++	–	6·51	+++	–	6·42	+	++	6·38	++	++	—
	$Na_3C_6H_5O_7$	15·4	6·97	40	6·63	+	–	6·63	+	–	6·56	–	++	6·46	+	+	—
24	$NaHCO_3$	3·0	7·07	25	6·89	–	–	6·82	–	–	6·79	–	–	6·60	–	+	—
	$NaHCO_3$	3·0	7·07	40	6·86	+	–	6·66	+++	–	6·60	–	++	6·45	++	+	Black spots
	Na_2HPO_4	4·9	6·90	25	6·71	–	–	6·66	–	–	6·60	–	+	6·46	–	+	Black film
	Na_2HPO_4	4·9	6·90	40	6·64	+	–	6·54	–	–	6·46	+	–	6·30	–	++	Black film
	$Na_3C_6H_5O_7$	9·2	7·00	25	6·94	–	–	6·81	–	–	6·74	–	++	6·62	+	++	Black film
	$Na_3C_6H_5O_7$	9·2	7·00	40	6·75	+	–	6·62	++	+	6·50	–	+++	6·38	–	++	Black film
25	$NaHCO_3$	5·0	7·20	25	6·83	–	–	6·96	+	–	6·78	–	+	6·62	+	+	Black spots
	$NaHCO_3$	5·0	7·20	40	6·80	+	–	6·93	++	–	6·57	–	+	6·49	+	++	Black spots
	Na_2HPO_4	8·15	6·92	25	6·60	–	–	6·75	+	–	6·51	+	+	6·41	–	++	Black spots
	Na_2HPO_4	8·15	6·92	40	6·53	+	–	6·65	+++	–	6·45	++	+	6·36	++	+++	Black film
	$Na_3C_6H_5O_7$	15·4	7·00	25	6·87	+	–	6·93	++	–	6·74	+	–	6·64	+	++	Black film
	$Na_3C_6H_5O_7$	15·4	7·00	40	6·67	++	–	6·79	++++	–	6·62	++	++	6·47	+	+++	Black film and spots

* The degrees of pitting and purpling reported in this table for the 7-months period represent the average results from three samples opened at the same time.

Tin sulphides may indeed be partially dissolved by the complex acids present, evolving some hydrogen sulphide. This will assist in the attack of steel to form iron sulphide, giving black particles in the cream; it may also collect in any gas space and give secondary bronzing or purpling on such tin as is out of contact with the cream salts and acids.

It is evident, therefore, that the production of sulphydryls during processing is a dominant factor in producing pitting after long storage as well as initial purpling. Such pitting as occurs in unpurpled cans is rarely serious in a storage period of some 7 months, though it may eventually become so.

(*b*) *Practical implications*

The practical implications of the experimental evidence of this and the previous paper in the commercial canning of cream should now be evident, but may be tabulated as follows:

(1) The cream to be processed must be derived from good milk. It is probable that abnormal milks, such as newly calved cow's milk or milk from animals suffering from mastitis, will tend to liberate sulphur compounds very readily on sterilization due to the abnormal condition of the protein phase.

(2) Only sound cans manufactured from good quality tinplate should be used. The danger of using cans in which much iron is exposed through exceptional porosity of the tin coating has been demonstrated.

(3) The minimum of stabilizer that will prevent coagulation of the cream should be used. The use of sodium bicarbonate seems preferable to disodium monohydrogen phosphate and trisodium citrate. The use of excess stabilizer has been shown to cause severe can attack.

(4) The minimum of heat treatment that will ensure the complete sterilization of the product should be used.

(5) Since, in general, the more serious defects are a product of time, the storage period should be as short as practicable.

If the above precautions are taken, there seems no reason why discoloration and corrosion in canned cream should not be eradicated.

Acknowledgements

This collaborative work between the Hannah Dairy Research Institute and the International Tin Research and Development Council has been initiated by the respective Directors of Research, Dr N. C. Wright and Mr D. J. Macnaughtan, to whom our thanks are due for facilities afforded and permission to publish.

References

(1) Jackson, Howat & Hoar (1936). *J. Dairy Res.* **7**, 284.
(2) Hunziker (1926). *Condensed Milk and Milk Powder.* Illinois, U.S.A.: published by the author.
(3) Hoar (1937). *Trans. Faraday Soc.* (in the Press).

(*MS. received for publication* 20 *June* 1937)

637.148

168. BITTERNESS AND THINNING IN CANNED CREAM

By A. A. NICHOLS, G. R. HOWAT and C. J. JACKSON

From the Hannah Dairy Research Institute, Kirkhill, Ayr

(With 2 Figures)

The development of a pronounced bitter flavour has been observed as one of the defects which may occur in canned dairy products during storage. Hammer (1) isolated from faulty evaporated milk an organism which he named *B. amarus*. Control cans of evaporated milk inoculated with this organism developed a bitter taste together with a slow-forming solid clot which was present after incubation for 89 days at 37° C. and at room temperature. An abnormal odour and an increase in acidity were characteristic of these conditions. Spitzer & Epple (2) investigated an extensive outbreak of bitterness in evaporated milk, though this was not accompanied by any change in the physical appearance of the product. They found that the milk was infected with a spore-bearing organism, similar to *B. panis* (Migula, 1900), which survived heating at 250° F. for 8 min. When the organism was inoculated into sterile milk it caused considerable degradation of the proteins producing large amounts of peptone substances and some ammonia; there was also an increase in acidity. Grindrod (3) also reports two heat-resistant organisms which were responsible for bitterness in evaporated milk. Although in the above investigations the causal organisms were spore-formers, Hunziker & Wright (4) isolated from coagulated and bitter evaporated milk some very small rod-like organisms with no spores which were unable to withstand heating at 212° F. for 15 min. Recently similar defects have been observed in canned cream. McMaster (5) reported both a marked bitter flavour and a water-like consistency in some defective batches of canned cream and was able to demonstrate that the trouble was caused by a heat-resistant bacillus, similar to *B. cohaerens*, which withstood 10 min. heating at 115·5° C.

During the bacteriological examination of faulty canned dairy products several batches of canned cream have been encountered in which the contents of the cans were exceedingly thin and had a bitter taste resembling quinine. Spore-forming organisms were isolated from the defective cans. It was also found that considerable breakdown of protein had occurred, as shown by an abnormally high proportion of acid-soluble nitrogen. No information was available as to the age of the batches or the conditions of manufacture and storage. Preliminary experiments showed that bitterness and thinning could be reproduced in normal canned cream by inoculation with the cultures iso-

lated from affected cream. It was decided to make a detailed study of the factors involved in the production of these two defects. The results of this study are dealt with under two heads, i.e. bacteriological and chemical.

Bacteriological studies

Three organisms have been isolated from defective batches of canned cream secured from different sources, i.e. cultures Nos. 6, 27 and 62. Each of these is capable of producing bitterness and thinning when inoculated into normal canned cream. The organisms belong to the aerobic spore-forming group of bacilli. In the past the differentiation and nomenclature of the aerobic spore-formers has been somewhat confused. According to the classification suggested by Ford (6) and his co-workers the organisms isolated are all similar to *B. vulgatus*, although No. 62 and probably No. 27 produce many mucoid smooth colonies and are therefore similar to the *B. subtilis* var. *viscosus* described by Ford. The Second International Microbiological Conference (1936) decided that Gottheil's strain of *B. subtilis* (or the so-called Marburg strain) should be the type culture of the aerobic spore-formers. Thus the organisms isolated should now be more correctly termed *B. subtilis*. In the differentiation of these organisms from other aerobic spore-formers reliance has been placed on the cultural characteristics suggested by Gibson (7) as being constant for the groups. Their characteristics are considered together, unless otherwise stated. All cultures were incubated at 37° C., since this appears to be their optimum growth temperature.

Morphological and cultural characteristics

Morphology. From broth, small rod-shaped organisms with rounded ends occur singly, in pairs and in short chains which seldom exceed five cells. In 12 hr. old cells there are often granules situated towards the extremities which disappear later and in no way resemble the granules of the *B. cereus* type. The cells from 24 hr. old agar slant cultures measure between 1·5 and 3·0μ, but are mostly 1·5μ long and about 0·5–0·7μ wide.

Motility. Young broth cultures are actively motile.

Staining properties. The three organisms are Gram-positive and are readily stained by the common dyes.

Spore formation. Spores are formed readily from about 12 hr. onwards on agar slants. Similar cells from 18–24 hr. old are nearly all sporing, but with age the spores become free and usually do not retain the old cell wall attached to them. Spores are also formed on other solid media and rapidly in broth scums. They are generally in a central position or towards one end of the cell. The cell wall which is very slightly enlarged by the spore does not usually exceed 0·8μ in width when grown on standard agar. The average width for young spores is 0·7μ and the length from 1·0 to 1·5μ.

Agar slants. There is abundant growth after 16 hr. incubation which with all cultures is easily brushed off with an inoculating needle. The growth of

No. 6 is raised and dull cream white to grey in colour with plumose undulating edge holding to the line of the inoculation neither wrinkled nor striated. It becomes drier and rather darker in colour with age. Cultures after several weeks at room temperature develop clear patches as though the organisms had disintegrated. The growth of No. 27 is not as dense as that of No. 6 and shows a greater tendency to spread. No. 62 grows less profusely, and with age becomes almost colourless transmitting light easily.

Milk-agar stab. Stab growth is only villous and scanty, but development is heavy on the surface after 1 day.

Milk-agar plates. After 24 hr. incubation surface colonies are 2–6 mm. in diameter, 3–4 mm. being the most usual size unless overcrowding occurs. Colonies tend to be circular but are spread if the agar is wet. The centres are dense and irregular in shape, do not transmit light, and are surrounded by a less dense granulated zone which is filamentous at the edges. Old surface colonies grow to 1 cm. in diameter and retain the dense dull centres surrounded by raised cream-coloured circular growth. As ageing occurs the colonies are surrounded by clear zones in the agar where the small amount of casein (1% separated milk) is digested. Subsurface colonies are irregular and dense and about 1 mm. in diameter at 24 hr. Later they may break through to the surface and develop into the type of colony already described. In addition No. 62 produces mucoid or smooth colonies.

Glucose-agar stab. One-day-old cultures show abundant surface growth which is finely wrinkled, dull and light brown in colour. On further incubation these wrinkles usually increase and become dark brown in colour, while a pink red colour develops under the surface of the agar. The degree of wrinkling and darkening varies considerably. Growth occurs down the slant to a considerable depth but is scanty. No. 62 has usually a smooth light-coloured surface growth which is viscous.

Gelatine stab. Liquefaction takes place readily at 22° C. After 3–5 days from one-third to one-half of the stab is liquid and a scum is formed which may be of rough or smooth appearance.

Broth. A heavy scum covers the surface after 18–24 hr. incubation. At first there is practically no turbidity or deposit until parts of the pellicle are dislodged. The scum may become wrinkled and have a "honeycomb" appearance although No. 27 usually remains smooth.

Potato. After 1 day a profuse folded or wrinkled growth covers the whole potato surface. Since the organisms grow closely together the folds become almost obliterated with age and at the same time the potato blackens and the culture darkens considerably.

Litmus milk. A soft coagulum is formed after 1–2 days and the milk clears rapidly from the surface downwards. All the curd is usually digested in from 3 to 6 days and sometimes digestion precedes clotting. As digestion advances the clear liquid at the surface becomes bright amber coloured, but after standing for some days the medium reverts to a port wine colour.

Other cultural characteristics. Further reactions brought about by the organisms during growth are shown in Table I.

Table I. *Cultural reactions of isolated strains*

Culture no.	Nitrate reduced to nitrite	Indol Ehrlich's method	Voges-Proskauer	Carbohydrate fermentation							Starch hydrolysis
				Levulose	Glucose	Saccharose	Lactose	Raffinose	Salicin	Mannite	
6	+	–	+	A	A	A	–	–	A	A	+
27	–	–	+	A	A	A	–	–	–	–	+
62	–	–	+	A	A	A	–	–	A	A	+

A = production of acid.

Heat resistance. Under laboratory conditions spore suspensions in sterile milk and cream have withstood temperatures up to 120° C. for as long as 40 min. The percentage survival shows, however, marked variations according to the age and condition of the culture. The factors influencing the heat resistance of these organisms are being further studied and will form the subject of a separate paper.

Inoculations into normal canned cream

In order to determine the conditions under which bitterness and thinning could be produced in canned cream, normal cans were inoculated with cultures of the above organisms.

Technique of inoculation and incubation. A number of cans of normal cream containing 23% butterfat were selected from the same batch manufactured at a local creamery. The cream was about 4 weeks old and had undergone the usual age thickening. The three organisms (i.e. Nos. 6, 27 and 62) were each inoculated from a 2-day-old culture into forty cans. The following method of inoculation was adopted. The outside of each can was thoroughly cleaned with alcohol, a small hole about $\frac{1}{8}$ in. in diameter was made in the top of the can with a sterile punch, and a loopful of the culture was introduced into the cream. The can was resealed by soldering over the hole a sterilized circular piece of tinplate about 1 in. in diameter. Using the same technique about twenty control cans were inoculated with a loopful of sterile water.[1] After inoculation the cans were well shaken before placing in incubators and were reshaken occasionally during storage. For examination of the cream, two cans of each batch were removed from the incubator at definite intervals.

Examination. The following routine examination was made on each can. Separate tubes of 1% glucose broth and litmus milk were each inoculated with 1 ml. and one loopful of the cream. These were incubated at 37° C. and were examined after 3 and 7 days for the presence of the organisms. Plate counts on the cream were carried out using 9 ml. saline dilution blanks and milk agar incubated at 37° C. for 3 days. Cream was also streaked directly on milk-agar plates and slides prepared for direct microscopic examination.

[1] The results of the control inoculations were uniformly negative, and are therefore not detailed in the text.

Production of bitterness and thinning. Table II shows the extent to which bitterness and thinning were produced by incubation at various temperatures. Only the results for cultures Nos. 6 and 62 are recorded, since these exhibited the widest differences in their action on the cream. Bitterness and thinning developed most rapidly with culture No. 6. All the cans incubated at 37° C. and most of those incubated at 32° C. were thin at the end of 2 weeks, while those incubated at 25° C. were just starting to lose their thick consistency, The cans kept at room temperature were practically unaltered. After 3–4 weeks

Table II. *Development of bitterness and thinning at different temperatures*

No. of days after incubation	Room temperature		25° C.		32° C.		37° C.	
	Flavour	Consistency	Flavour	Consistency	Flavour	Consistency	Flavour	Consistency
				Culture No. 6				
5	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Normal	Normal	Normal	Normal	Normal	Slight thinning	(1) Very bitter, (2) bitter	Thin
15	Normal	Normal	Normal	Slight thinning	(1) Slight putrefaction, (2) bitter	Thin	(1) Slight putrefaction, (2) bitter	Thin
22	Bitter	Thin	Bitter	Thin	Very bitter	Very thin	(1) Bitter, (2) very bitter	Very thin
54	—	Thin	—	Thin	—	—	—	—
				Culture No. 62				
4	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Normal	Normal	Normal	Normal	(1) Slight putrefaction, (2) bitter	Slight thinning	Slight putrefaction	Slight thinning
21	Normal	Normal	Normal	Normal	Slight bitter	Thin	(1) Normal, (2) slight bitter	Thin
53	Normal	Normal	—	Thin	(1) Very bitter, (2) bitter	Thin	—	—

Note. In each instance two duplicate cans were examined. If the results differed the cans have been recorded separately.

cans stored at 32 and 37° C. were very thin, while those kept at 25° C. and at room temperature were thin. The development of bitterness usually, but not invariably, ran parallel with the thinning. The results for cans inoculated with culture No. 62 were similar throughout the periods of incubation, but the development of both bitterness and thinning was less rapid. Thus at the end of 3 weeks no abnormality was observed in cans kept at 25° C., while at room temperature the cans were still unaffected after 5½ weeks' storage.

Plate counts of inoculated cans. The organisms were at all times recoverable from the inoculated cans in pure culture. After about 6 weeks their presence could not be detected in films made directly from the cream, nor was it possible

except in the early stages to demonstrate the persistence of the bacilli by streaking on agar plates. Small inoculations of the cream (one loopful) into litmus milk and glucose broth only produced growth in the early stages of the experiment but with larger inoculations (1 ml.) growth could be produced at all stages. The necessity for using such large inoculations for the recovery of these organisms from the cans is further illustrated by the results of plate counts. Table III shows the counts of inoculated cans after incubation at 37° C. It is remarkable to find that bitterness and thinning can be produced in

Table III. *Plate counts of inoculated cans after incubation at* 37° C.

Culture No. 6		Culture No. 27		Culture No. 62	
No. of days of incubation before testing	Mean of plate counts of 2 cans opened on same date	No. of days of incubation before testing	Mean of plate counts of 2 cans opened on same date	No. of days of incubation before testing	Mean of plate counts of 2 cans opened on same date
7	46,000	10	1,000	5	140,000
14	2,400	17	180	12	500
21	99,000	24	180	19	70
28	38,000	31	780	26	110
35	6,900	38	70	33	130
42	650	45	30	40	160
49	70	52	90	47	240
56	720	59	20	54	120
77	3,550	82	60	66	140
143	2,900	150	—*	124	20
154	2,190	171	10	158	280
167	—	175	40	—	—

* None found in 1/10th dilution.

cream having such low numbers of viable organisms. The counts of such organisms after different periods of incubation show, moreover, unexplained variations which could not be correlated with the intensity of bitterness or the extent of development of thinning. This suggests that such defects may be partly caused by the action of extracellular bacterial enzymes. It may be mentioned that in only three instances were viable organisms recovered from the cans other than those originally used for the inoculations. Casual contamination of the cans with extraneous organisms was therefore very infrequent.

Chemical studies

During the foregoing investigation opportunity was taken to study in detail the chemical and physical changes accompanying the development of bitterness and thinning in the canned cream. For this purpose the following determinations were made: titratable acidity, *p*H, viscosity, and the distribution of protein and protein degradation products.

Methods of determination

Titratable acidity and pH. 10 ml. of the cream[1] were diluted with an equal volume of distilled water and after mixing titrated with $N/10$ NaOH. The results are expressed as ml. of alkali required for neutralization. pH was measured by quinhydrone electrode at $16 \pm 1°$ C.

Viscosity. It was found that measurements of viscosity by a torsion method gave unsatisfactory results owing to the wide variations in the viscosity of the creams. The following direct method was therefore employed. Twenty ml. of the cream were measured into the bottom of a large Ostwald viscometer. The bulb at the end of the capillary tube of this instrument had a capacity of approximately 15 ml. The viscometer was placed in a water-bath at 25° C. and allowed to come to temperature. The arm containing the capillary tube was then connected by pressure tubing, provided with a clip, to a 5 l. vacuum flask. This flask in turn was connected to a vacuum pump and mercury manometer. The clip between the viscometer and the vacuum flask having been closed, the pressure in the flask was lowered by means of the vacuum pump until it reached 10″ less than atmospheric pressure. The clip between the flask and the vacuum flask was then closed and that between the flask and the viscometer opened, and the cream was thus forced up the capillary tube into the top bulb. The time required for the filling of the bulb by the cream was noted by stopwatch. Since all other factors were kept constant throughout the series the viscosity of the cream was directly proportional to its time of flow through the capillary. No calculations of apparent viscosity have been made and the results are therefore expressed in units of time.

Protein precipitations. Total non-protein nitrogen was determined by trichloroacetic acid precipitation by Sanders's method (8). A second quantity of cream was used for determining peptone and subpeptone nitrogen. A known weight of the cream (roughly 10 g.) was weighed into a 50 ml. standard flask and diluted with about 20 ml. of water. Six ml. of 25% sulphuric acid were then added, followed by 4 ml. of 10% phosphotungstic acid with vigorous shaking. The contents were made up to the mark and allowed to stand for 12–24 hr. before filtering.

From these two filtrates the non-protein nitrogen and subpeptone nitrogen were obtained: the peptone nitrogen was calculated by difference. By determining the total nitrogen of the cream the total protein was also ascertained. All nitrogen determinations were made by the micro-Kjeldahl method.

Lactose was estimated by the Fehling-Soxhlet method on a tungstic acid filtrate. Since no significant variations in the amount of lactose occurred during the experiment the results have not been recorded.

[1] It was found impracticable to measure out definite volumes of cream with an ordinary pipette owing to the high viscosity of many of the samples. It was found, however, that a hypodermic syringe and needle could deliver any required volume rapidly and with a fair degree of accuracy.

Discussion of results

The results of the examination of the inoculated cans over a period of 6 months are shown in Tables IV–VI. It will be convenient to discuss these results under four heads.

Progressive changes during incubation. It has already been noted that the cans used throughout the investigation were obtained from a single batch of normal canned cream. Nevertheless, considerable variations were found in the chemical composition of the contents of duplicate cans. A similar individuality among the cans was observed in their susceptibility to attack by the inoculating organisms. The reproduction of exact duplicates in any biological investigation cannot be expected. The lack of consistency in duplicate samples was not, however, sufficient to mask the general trend of the changes which took place during incubation. These consisted of the gradual production of bitterness and thinning (the latter being clearly shown in the viscosity determinations) and the progressive breakdown of protein, as indicated by the increase in the non-protein nitrogen and the peptone and subpeptone fractions. There was also an increase in titratable acidity and a slight decrease in the *p*H of the cream during incubation. It may be noted that the breakdown of protein varied with the culture employed. It was most rapid and proceeded farthest with culture No. 6, and was slowest with culture No. 62. These differences ran roughly parallel to the development of bitterness and thinning, a point which has already been noted in regard to the development of these defects during storage, as shown in Table II.

It should be mentioned that a putrefactive odour was sometimes noticed on opening certain cans, and a corresponding flavour was apparent in the cream. Plating of these samples failed, however, to reveal any viable organisms other than those originally inoculated into the cans.

Relation of protein breakdown to the development of bitterness. The variability of the results shown in Tables IV–VI makes it difficult to draw any strict comparison between the extent of protein breakdown and the development of bitterness. By regrouping these results on the basis of flavour, as in Table VII, it has, however, been possible to show whether bitterness is directly related to protein degradation. In drawing up this table, all samples in which a putrefactive smell or taste was found have been relegated to a separate group. The close association between the development of bitterness and the breakdown of protein is clearly demonstrated in this table, the progressive increase in bitterness being strictly paralleled by an increase in the non-protein nitrogen and in the peptone and subpeptone fractions. Considering the difficulty involved in an accurate assessment of flavour, it is, too, remarkable to find such relatively small standard deviations within each group. The differences between the groups are, moreover, with one exception[1] significant. The group in which

[1] I.e. the difference in the subpeptone nitrogen fractions of the "bitter" and "very bitter" samples.

Table IV. *Effect of incubation at* 37° C. *with Culture No.* 6

Can no.	Period of incubation, days	General condition of cream	Titratable acidity (ml. of *N*/10 alkali)	*p*H	Viscosity (time of flow) min.	sec.	Total N (mg of N in 100 g.)	Expressed as percentage of total N in sample: Total protein N	Non-protein N	Peptone N	Sub-peptone N
Control	0	Normal	—	—	25	0	399·0	93·3	6·6	1·6	5·0
Control	0	Normal	—	—	25	0	371·4	89·1	10·8	4·9	5·9
1	7	Slightly bitter; slightly thin	1·80	6·58	15	31	295·0	71·8	28·1	18·3	9·8
2	7	Slightly bitter; slightly thin	1·80	6·61	15	26	261·1	67·2	32·8	20·7	12·1
3	14	Slightly bitter; thin	2·00	6·54	11	16	392·6	70·2	29·8	20·9	8·9
4	14	Slightly bitter; slightly thin	2·05	6·53	12	52	342·0	67·4	32·6	23·2	9·4
5	21	Bitter; thin	2·40	6·49	6	47	460·9	68·2	31·9	21·8	10·1
6	21	Bitter; thin	2·10	6·49	3	53	407·5	57·3	42·7	30·6	12·1
7	28	Bitter; very thin	2·60	6·42	1	14	386·7	44·0	55·9	37·0	18·9
8	28	Bitter; very thin	2·45	6·42	—		383·8	49·6	50·4	31·2	19·3
9	35	Bitter; slight putrefaction; very thin	4·05	6·31	0	56	404·6	19·65	80·4	35·7	44·7
10	35	Slightly bitter; slight putrefaction; thin	2·30	6·43	8	3	396·3	61·9	38·2	33·2	5·0
11	42	Bitter; thin	3·00	6·39	2	23	396·4	45·4	54·6	36·9	17·8
12	42	Bitter; thin	2·95	6·38	1	51	408·2	46·7	53·3	35·2	18·1
13	49	Bitter; slight putrefaction; thin	3·55	6·29	1	13	383·0	32·6	67·4	45·1	22·3
14	49	Bitter; thin	3·05	6·38	2	14	354·7	42·7	57·3	26·9	30·4
15	56	Bitter; slight putrefaction; thin	2·90	6·42	1	32	387·4	38·2	61·8	31·4	30·4
16	56	Bitter; putrefaction; thin	3·55	6·27	1	22	413·7	26·8	73·2	51·4	21·8
17	77	Bitter; thin	3·00	6·55	1	30	383·7	52·7	47·3	14·8	32·6
18	77	Very bitter; thin	3·45	6·40	1	10	416·7	36·3	63·6	37·1	27·2
19	143	Very bitter; slight putrefaction; very thin	3·40	6·27	1	15	423·5	32·5	67·5	39·3	28·2
20	143	Very bitter; lumpy; very thin	3·90	6·23	0	59	422·2	26·0	74·0	43·5	30·6
21	154	Bitte.; slight putrefaction; thin; lumpy	4·00	6·25	1	21	434·6	28·8	71·2	41·7	29·5
22	154	Very bitter; putrefaction; thin; smooth	4·10	6·20	1	19	411·6	22·0	78·0	46·5	31·5
23	167	Bitter; slight putrefaction; thin; lumpy	3·60	6·29	0	48	406·2	43·2	56·8	38·5	18·3
24	167	Very bitter; thin; smooth	4·30	6·71	0	50	411·8	16·6	83·4	55·0	28·4

Table V. *Effect of incubation at* 37° C. *with Culture No.* 27

Can no.	Period of incubation days	General condition of cream	Titratable acidity (ml. of *N*/10 alkali)	*p*H	Viscosity (time of flow) min.	sec.	Total N (mg of N in 100 g.)	Expressed as percentage of total N in sample: Total protein N	Non-protein N	Peptone N	Sub-peptone N
Control	0	Normal	—	—	25	0	399·0	93·3	6·6	1·6	5·0
Control	0	Normal	—	—	25	0	371·4	89·1	10·8	4·9	5·9
1	10	Slightly bitter	2·00	6·52	12	25	382·3	77·7	22·3	12·4	9·9
2	10	Normal	1·40	6·75	18	0	370·9	89·2	10·8	15·3	5·5
3	17	Slightly bitter	1·70	6·62	16	29	377·6	74·5	25·5	12·5	13·0
4	17	Bitter; thin	2·40	6·52	4	35	401·7	57·7	42·3	33·3	9·1
5	24	Slightly bitter; thick; small lumps	1·55	6·69	23	29	397·7	83·0	17·0	11·0	6·0
6	24	Slightly bitter; thick	1·90	6·55	16	27	402·0	74·0	26·0	16·7	9·3
7	31	Bitter; very thin	2·85	6·38	2	14	415·2	48·0	52·0	34·3	17·7
8	31	Bitter; very thin	2·95	6·40	1	11	408·5	39·8	60·2	38·9	21·3
9	38	Slight putrefaction; lumpy	2·30	6·07	21	32	381·1	81·4	18·6	9·8	8·7
10	38	Slightly bitter; slight putrefaction	3·20	5·73	10	52	399·8	70·1	29·9	19·3	10·6
11	45	Slightly bitter; lumpy	2·40	6·42	5	2	377·8	58·5	41·5	26·3	15·2
12	45	Slightly bitter; thin; lumpy	2·45	6·43	5	42	384·0	62·1	37·9	25·1	12·9
13	52	Slightly bitter; thin; lumpy	2·05	6·58	—		427·7	80·0	20·0	2·1	17·9
14	52	Slightly bitter; very thin	2·75	6·51	1	18	408·2	53·7	46·3	34·0	12·3
15	59	Bitter; putrefaction; thin; lumpy	2·70	6·40	1	12	375·7	39·1	61·0	29·3	31·7
16	59	Bitter; putrefaction; thin; lumpy	3·65	6·26	1	40	390·5	23·8	76·3	56·8	19·5
17	82	Slightly bitter; putrefaction; thin	2·40	—	8	46	392·8	62·5	37·4	12·6	24·8
18	82	Very bitter; thin; lumpy	3·90	—	—		395·5	33·9	66·1	55·0	11·1
19	150	Putrefaction; thin; lumpy	3·55	5·93	2	20	417·9	36·9	63·1	43·2	19·8
20	150	Putrefaction; thin; lumpy	3·20	6·42	11	31	339·8	54·0	46·0	32·2	13·8
21	171	Bitter; thin	3·90	6·05	1	14	429·2	25·2	74·8	49·3	25·5
22	171	Bitter; thin	—	6·30	0	57	404·8	31·0	68·9	50·9	18·1
23	175	Slightly bitter; very thin; few lumps	3·00	6·45	1	53	428·0	42·3	57·7	39·1	18·6
24	175	Very bitter; thin	3·50	6·39	1	19	416·9	28·7	71·3	43·6	27·7

Table VI. *Effect of incubation at* 37° C. *with Culture No.* 62

Can no.	Period of incubation days	General condition of cream	Titratable acidity (ml. of *N*/10 alkali)	*p*H	Viscosity (time of flow) min.	sec.	Total N (mg of N in 100 g.)	Expressed as percentage of total N in sample: Total protein N	Non-protein N	Peptone N	Sub-peptone N
Control	0	Normal	—	—	25	0	399·0	93·3	6·6	1·6	5·0
Control	0	Normal	—	—	25	0	371·4	89·1	10·8	4·9	5·9
1	5	Normal	1·50	6·59	26	27	373·6	87·9	12·1	5·6	6·5
2	5	Normal	1·30	6·76	29	32	374·2	92·1	7·9	1·5	6·4
3	12	Normal	1·65	6·65	23	44	392·8	82·8	17·4	9·8	7·6
4	12	Normal	1·60	6·67	25	53	393·8	83·2	16·8	10·8	6·0
5	19	Normal	1·55	6·70	20	8	378·8	87·6	11·8	5·5	6·3
6	19	Bitter; slight putrefaction; thin	2·20	6·61	4	41	397·2	66·7	33·3	20·6	12·7
7	26	Normal	1·45	6·61	—		389·3	86·5	13·3	7·7	5·7
8	26	Bitter; thin	2·00	6·48	6	36	409·8	64·4	35·6	25·3	10·3
9	33	Slight putrefaction; normal	1·85	6·62	13	18	390·2	73·1	26·9	17·1	9·8
10	33	Bitter; normal thickness	1·90	6·62	14	29	384·5	69·9	30·0	20·0	10·0
11	40	Normal	1·90	6·55	17	58	398·6	73·3	26·7	17·7	9·0
12	40	Normal	1·90	6·50	17	17	393·8	72·7	27·3	18·7	8·6
13	47	Bitter; very thin	2·60	6·48	1	24	383·3	42·2	58·0	49·8	7·9
14	47	Normal	1·90	6·65	26	1	342·9	72·7	27·3	6·3	21·0
15	54	Slightly bitter; slight putrefaction; slightly thin; lumpy	2·40	6·49	5	35	403·5	65·8	34·3	22·1	12·2
16	54	Slightly bitter; slight putrefaction; slightly thin; lumpy	2·20	6·54	6	24	399·8	63·8	36·2	23·2	13·0
17	66	Slightly bitter; thin; lumpy	2·60	6·43	2	7	387·4	51·7	48·5	34·4	14·1
18	66	Normal	1·90	6·44	19	1	409·9	76·8	23·7	15·7	8·0
20	124	Bitter; very thin	3·20	6·38	1	12	407·8	37·2	62·8	40·8	22·0
23	158	Very bitter; very thin	3·70	6·24	1	30	427·1	29·9	70·2	58·7	11·4
24	158	Slightly bitter; thin; lumpy	2·10	6·31	22	3	422·3	62·9	37·1	26·5	10·6

putrefactive flavours and odours were found shows on the whole very much larger standard deviations, and the mean values for this group fall close to the mean values for the whole series of samples examined. The development of such putrefactive changes does not therefore appear to be directly related to the onset of bitterness.

It is not possible to determine from the results what is the actual cause of the bitter flavour, or whether it is associated with the peptone or subpeptone fractions. It is, however, clear that the development of bitterness is closely related to the general breakdown of the protein present in the cream. It should, perhaps, be mentioned that tests carried out on samples of normal and defective creams failed to reveal any change in either the lactose or the fat.

Table VII. *Relation of protein breakdown to bitterness and thinning*

	Protein breakdown products (expressed as percentage of total N in sample)									Viscosity (time of flow in min.)		
	Non-protein N			Peptone N			Subpeptone N					
Flavour	Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.
Normal	16·7	7·3	2·1	9·7	5·8	1·7	8·1	4·2	1·2	22·8	4·2	1·2
Slightly bitter	34·8	10·5	2·7	22·3	8·3	2·1	12·5	4·4	1·1	11·3	6·7	1·7
Bitter	53·6	13·5	3·2	35·1	11·2	2·6	18·6	7·6	1·8	3·1	3·3	0·8
Very bitter	72·4	6·2	3·1	47·8	8·0	4·0	25·0	7·1	3·6	1·2	0·4	0·2
Putrefaction	51·9	19·3	4·7	32·4	11·4	2·8	19·7	10·1	2·5	5·5	5·8	1·4
Mean for all samples	43·7	20·9	2·5	27·9	14·5	1·8	16·1	8·8	1·1	8·9	8·8	1·1

Relation of protein breakdown to thinning. It will be noted from Table VII that the production of bitterness and thinning run parallel. It would therefore be expected that thinning would also be associated with the breakdown of protein. This fact is confirmed by a direct examination of the relationship between thinning (as measured by viscosity) and the non-protein nitrogen. Fig. 1 illustrates this relationship in the form of a scatter diagram, in which the viscosity is plotted against the non-protein nitrogen. In this diagram the results for the three cultures have been combined. The relationship is shown in quantitative form in Table VIII, where the correlation coefficients between viscosity and non-protein nitrogen are recorded separately for each culture. Owing to the non-linear relationship which is apparent from Fig. 1, the correlation ratios (η) have also been calculated. The figures show close agreement with the correlation coefficients. The results show clearly that the reduction in viscosity is directly related to the proportion of the protein which is broken down by bacterial action.

Table VIII. *Correlation coefficients (r) and correlation ratios (η) between viscosity and non-protein nitrogen*

Culture	r	S.E.	η
No. 6	0·81	±0·127	0·88
No. 27	0·86	±0·060	0·96
No. 62	0·90	±0·098	0·86

The relation of the physical structure of cream to its viscosity. The thinning of canned cream by bacterial action provided a unique opportunity of studying the relation of the physical structure of cream to its viscosity. The original cream was so thick that a teaspoon would stand almost stationary in it; after inoculation and a prolonged period of incubation the cream had become so thin that it resembled milk. Yet both products contained the same proportion of fat, namely 23%. The only detectable change in the thinned cream was, as noted above, the breakdown of the protein.

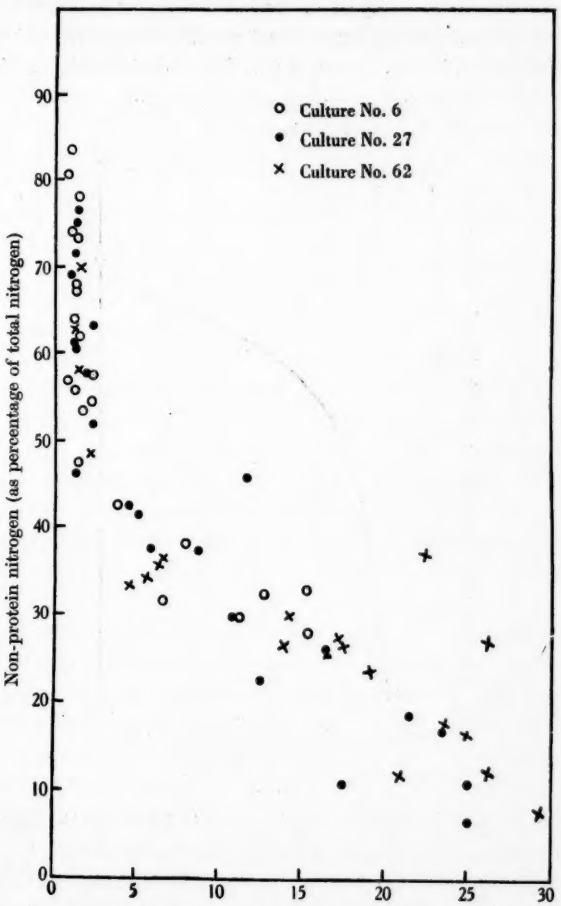


Fig. 1.

Among the methods which have been suggested for investigating the internal structure of liquids is that of determining the relation of the time of flow through a viscometer to the shearing force applied. Since viscosity depends on the relative motion of contiguous portions of the fluid, any difference in

internal structure should be revealed by this means. The viscosity (time of flow) of samples of normal (i.e. thick) cream and of bitter (i.e. thinned) cream was therefore determined by the method already described, but shearing forces varying from 5 to 30 in. pressure of mercury were employed. The results are shown in Fig. 2. It will be seen that, although the rates of flow differed greatly for the two types of cream (the thick cream (crosses) giving figures about eight times as large as the thinned cream (circles)), the general shape of the two curves was practically identical. It seems unlikely that there could be such a marked similarity in response to variations in shearing force unless the two types of cream possessed the same general structure. This was borne out by a microscopic examination of the fat phase: it was found that the degree of clumping

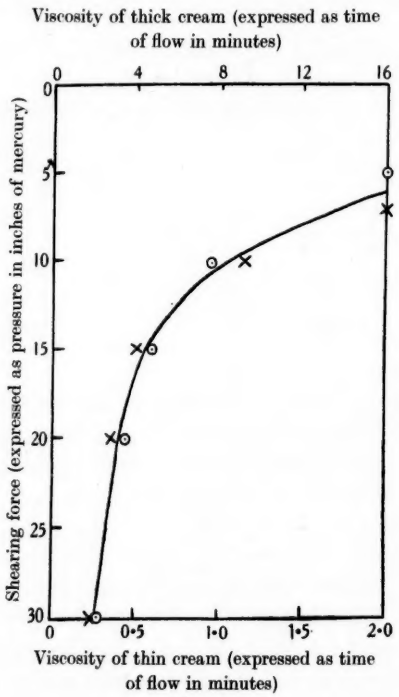


Fig. 2.

of the fat globules (which is held by Babcock (9) to be one of the most important factors which affects the viscosity of cream) was similar in both types of cream. It appears safe to conclude that *in the present instance* the viscosity was affected to a greater extent by alterations in the composition of the continuous (liquid) phase, which contains the protein, than by alterations in the distribution or extent of clumping of the fat phase.

Application of the results in commercial practice

Owing to the high thermal death-point of the aerobic spore-forming organisms which cause bitterness and thinning in canned cream it may be extremely

difficult to control this defect under commercial conditions. It has already been noted that spore suspensions have withstood temperatures up to 120° C. for as long as 40 min. The thermal death-point will, however, depend largely on the age of the spore cultures and possibly on certain environmental factors such as acidity. Less drastic heat treatment may therefore be sufficient to kill off the organisms in commercial practice. There is, of course, a limit to the extent of heat treatment to which cream can be subjected during processing: too high a temperature or too long a period of heating will result in loss of stability and in the production of an undesirable burnt flavour. Moreover, even if the thermal death-point was known and the cream could be safely subjected to the necessary heat treatment, it must be borne in mind that the temperature of the sterilizer does not give a true indication of the temperatures inside the cans. Loveless (10) has shown that there is a marked lag in the attainment of the required temperatures by the contents of cans of cream. It appears, therefore, that in practice the prevention of bitterness and thinning must depend more on improving the quality of the incoming milk supply than on altering the conditions of sterilization of the canned product.

A second point of importance in the control of bitterness is the identification of defective cans of cream before despatch from the creamery. It is apparent from the results detailed in Table II that the commercial practice of incubating canned cream for not more than 2 weeks at temperatures of about 75–85° C. would frequently be insufficient to reveal the defect. It appears desirable, particularly where there is any history of this defect at a creamery, to store the canned cream for a further period of, say, 4 weeks or more at room temperature in order that the defect may be sufficiently developed in any affected cans to enable these to be picked out. In this connexion it may be noted that thinning almost invariably accompanies bitterness, and this therefore provides a simple method of identifying the defect without opening the can.

It is, of course, desirable that there should be a careful bacteriological control of all batches of cream where laboratory facilities are available for this purpose. It has been pointed out, however, that if small inoculations of cream are used (for example, a single loopfull) there is a serious risk that the presence of viable organisms will be missed. At least 1 ml. of cream should be used for such inoculations. On the other hand, the isolation of organisms of *B. subtilis* type from canned cream should not be taken as indicating that the cream will inevitably become bitter. In an extensive examination of samples of canned dairy products some thirty-six strains of *B. subtilis* have been isolated, and these all showed the same morphological and cultural characteristics as strains which produce bitterness and thinning. Yet only a very few of these thirty-six strains were capable of producing the defect when inoculated into normal cream. It appears that the only certain method of identifying the typical organism is by its ability to produce bitterness and thinning in the normal product.

Summary

1. Three organisms have been isolated from defective commercially-canned cream which, on inoculation into normal cream, cause bitterness and thinning.

2. These organisms are described and are shown to be strains of *B. subtilis*. Their spores are capable of withstanding temperatures up to 120° C. for as long as 40 min.

3. The rate of development of bitterness and thinning in experimentally inoculated cans is shown to vary with the culture used and with the temperature of incubation.

4. Both bitterness and thinning are associated with the breakdown of protein, as indicated by determinations of the non-protein nitrogen and of the peptone and subpeptone fractions.

5. The alterations in viscosity do not appear to be due to any fundamental change in the structure of the cream, nor to differences in the degree of clumping of the fat globules.

6. The applications of the results in commercial practice are discussed.

Acknowledgements

The authors desire to express their thanks to the Scottish Milk Marketing Board for furnishing the necessary supplies of normal canned cream, and to the various firms from whom defective samples have been obtained. They are also indebted to Dr T. Gibson for his help in classifying the organisms, to Dr James Wilkie for his assistance in the statistical treatment of the results, and to Dr N. C. Wright for his constant advice throughout the work and in the preparation of this paper.

REFERENCES

(1) Hammer (1919). *Res. Bull. Ia agric. Exp. Sta.* No. 52.
(2) Spitzer & Epple (1920). *J. Dairy Sci.* **3**, 486.
(3) Grindrod (1928). *Canning Age*, **9**, 692.
(4) Hunziker & Wright (1910). *Bull. Ind. agric. Exp. Sta.*
(5) McMaster (1932–3). *Rep. Fruit Veg. Pres. Sta., Campden*, pp. 71–4.
(6) Ford (1916). *J. Bact.* **1**, 273, 493.
(7) Gibson (1937). *Proc. Soc. agric. Bact. Edinb.*
(8) Sanders (1923). *J. Ass. off. agric. Chem.* **16**, 140.
(9) Babcock (1931). *Tech. Bull. U.S. Dep. Agric.* No. 249.
(10) Loveless (1935). *Food*, **5**, 52.

(*MS. received for publication* 12 *August* 1937)

636.2:612.664.31:575

169. A STATISTICAL INQUIRY INTO THE INHERITANCE OF MILK YIELD IN THREE HERDS OF DAIRY SHORTHORN CATTLE

By A. D. BUCHANAN SMITH, M.A., B.Sc. (Agr.), M.S.A.
University of Edinburgh, Institute of Animal Genetics

(With 1 Figure)

CONTENTS

	PAGE
Part I. The inquiry and its results	347
Part II. Detailed examination of the results	352
Part III. Review and conclusions	359
Summary	362
Appendix I. Tables of relationship groups, etc.	363
Appendix II. Summary of all results on the subject	367
References	368

Part I. The inquiry and its results

Under the best of circumstances it is seldom easy to trace the mode of the inheritance of a quantitative character. When the expression of that character is also conditioned by environmental factors, the difficulties increase. A slow reproductive rate further retards the inquiry, but perhaps the crowning tribulation is when the expression of the character is restricted to one sex. Such are the troubles that afflict an investigation into the inheritance of milk yield in cattle. And they are further magnified when it is remembered that milking capacity is not a unit character, but comprises many facets which are themselves both quantitative and qualitative.

Yet the fact that the mode of inheritance may be a complicated one is no argument against the importance of the genetic basis in the manifestation of that character. That, in pre-Mendelian days, we understood nothing concerning heredity did not diminish the part it played. It is, however, occasionally argued that if a character is transmitted in a complicated manner then, even supposing we obtain a full knowledge of the mode of its transmission, it will not be possible to use that knowledge to practical advantage. There is some truth in such an argument, but there are plenty of instances to the contrary. In fact it is neither possible nor reasonable for us to say that knowledge, at present hidden, will, when discovered, be of no practical use. In the history of Science there are many examples where an apparently useless piece of knowledge has been discovered to be the essential clue to the solution of some practical problem.

This argument is not infrequently used when an attempt is made to apply the principles of genetics to the productive qualities of slow-breeding animals which must, a priori, be dependent upon a multitude of genes, the cumulative effect of which gives the appearance of a kind of blending inheritance. But if sex-linked genes—or even autosomal genes which act in an inhibitory manner—in any way condition the expression of the character, then, if they are of major importance, it is essential that allowance be made for them, for they must affect the practical considerations involved.

It is easy to select for a character inherited as a simple recessive. As regards other kinds of autosomal inheritance (dominance, incomplete dominance, or multiple factor), improvement can be fairly easily effected by the intelligent use of the Progeny Test. But with sex-linked genes the Progeny Test, while it may allow for a certain measure of improvement, is but a slight improvement upon the existing method of mass selection.

The principal obstacles which beset a study of the inheritance of milk yield have never really been appreciated. In the first place milk yield, which is itself a quantitative characteristic, must be dependent for its expression upon a number of genes. Multiple factors must be involved since genes which do not affect the organs of milk secretion may still have greater or less effect upon the yield. It may be argued that a few factors primarily affecting the organs of milk secretion are mainly responsible. However, as expression of such genes as increase milk yield may be inhibited by other genes which influence other parts of the body of the cow, the general effect is bound to be one of multiple factors. Nevertheless it may be possible, with suitable material, to discover the mode of transmission of a few of these major factors.

The second obstacle is the effect of environment upon yield, and the difficulty of getting figures in which the differences can be fairly interpreted as being primarily of genetic origin. Of all the obstacles this has been the one which has been most clearly recognized. Many studies, such as those of Gowen(1), have been made with very large numbers in the hope that, by this means, environmental effect would be evened out. Von Patow(2) has made a noble effort to overcome this by means of his "byre-average", whereby all the yields recorded in any one year are corrected by a comparison with the yields in other years. There is, however, a grave danger lest the adoption of such a correction factor flatten out the genetic influences which are at work.

The third difficulty is that the yield of milk is limited to one sex only. Therefore correlations with male relatives are impossible. Yet if a study of variation is to be made, such a comparison is indispensable, both from a scientific and a practical point of view. Unless the influence of the different parents and grandparents can be measured on a comparable basis, it is not possible to arrive, by means of the statistical method, at any conclusion whatsoever concerning the mode of the inheritance of milk yield: nor is any practical information available as to the relative importance of different ancestors in the pedigree of any animal. To meet this difficulty methods of

"indirect correlation" have been used first by Gowen (1) and then by Gifford & Turner (3), Buchanan Smith (4) and others. But these proved unsatisfactory for various reasons, particularly that the indirect correlations to male relatives could not be compared to the direct correlations to female relatives.

To overcome this difficulty another method, devised by Dr R. A. Fisher, has been already employed by the present writer (5). Briefly the argument is as follows: the closer the blood relationship of two animals, the more genes they should have in common, the more alike they should be, and, in the case of dairy cattle, the less difference there should be between their milk yields. The difference between the yields of any two cows may be measured by subtracting the lower from the higher yield and squaring the difference. Thus the difference between cows, according as they are differently related, may be compared. The unit of calculation is a pair of cows similarly related.

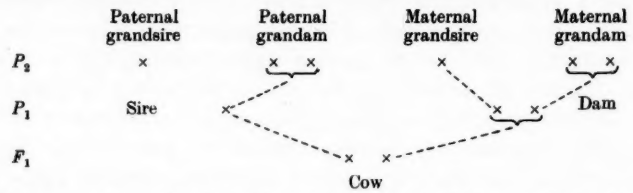
In the first study by this method there were 200 pairs of full sisters, that is to say each cow of a pair had the same sire and the same dam. There was, of course, a diversity of sires and dams, but the link binding each pair of cows was the fact that they both possessed the same sire and the same dam. The squared differences for each pair were then added together and divided by the number of pairs, and this figure, $\Sigma D^2/N$, was taken as the index of variability which occurred in the yields of animals thus related. The squared difference of a random sample of pairs of unrelated cows was taken as the basis of comparison, since this could be assumed to be 100% variability within the group studied. The figures previously obtained (5) are shown in Table I.

Table I. *Differences in milk yield and butterfat yield of pairs of Ayrshire cows according to their relationship classification*

	Number of pairs N	Milk yield $\Sigma D^2/N$	Butter fat yield $\Sigma D^2/N$
(1) Full sisters	200	24,822	4150
Half sisters:			
(2) By same sire with dams paternal half sisters	2027	27,821	4545
(3) By same sire with dams unrelated	6361	35,698	4973
(4) By same dam with sires unrelated	158	32,837	4358
Cousins:			
(5) By sires which are full brothers with dams unrelated	415	55,920	4742
Half cousins:			
(6) Same paternal grandsire	6435	49,931	6435
(7) Same maternal grandsire	2303	24,218	6576
(8) Same paternal grandam	1166	44,128	6036
(9) Same maternal grandam	185	33,191	5389
Unrelated pairs:			
(10) Random sample	6660	45,579	7950

An examination of this table shows that, as the degrees of kinship widen, the figures representing the variability in milk yields also increase, with one or two exceptions. The figures for butterfat yield show a remarkably steady increase. The basal figure for comparison is that of the unrelated pairs (Class 10) and, as regards total yield of milk, this is 45,579. Two of the figures for the

related groups (Classes 5 and 6) exceed this, and these are the figures for paternal full cousins and for half cousins whose only near ancestor in common is the paternal grandsire. The figure for paternal full cousins is based on small numbers, and is perhaps somewhat biased, due to the fact that a large number of pairs in this group traced to the same full brothers. The other group which shows extreme variation is the group whose only near ancestor in common is the paternal grandsire. This fact, together with the fact that the figure (Class 7) for half cousins tracing to the same maternal grandsire is lower than was anticipated, has been explained as possibly being due to the fact that some of the factors governing the inheritance of milk yield are transmitted in a sex-linked manner. If this were the case, then the influence of the paternal grandsire is much diminished, as the following diagram shows:



The fact that the method employed is sound may be seen from the similar figures obtained for yield of butterfat which are placed in the third column, and which show a fairly consistent increase in variation as the degrees of kinship widen, culminating in the highest figure for pairs of unrelated cows.

The data on which these figures were based suffered from certain defects, which indeed were common to all previous studies by the correlation method. In the first place the data consisted of the published records of cows. Since low records are omitted from publication, it was considered possible that this might account, in some undefinable manner, for the fact that the maternal grandsire appeared to exert a greater influence on his granddaughters than did the paternal grandsire.

By confining this study of Ayrshire cows to thirty herds in the same part of Scotland, and all managed under similar conditions, it was hoped that the force of environmental factors would be lessened. Despite the fact that this was more than any previous investigator by the statistical method had attempted, it was felt that the obstacle of environment had not been adequately overcome, and that a better attempt to do so might be made.

Accordingly, the next investigation was confined to three large herds of Dairy Shorthorns, and each herd was treated as a unit, only combining them for the final analysis. Thus the records of each cow in a pair were made under practically identical circumstances. Where more than four years elapsed between the record of one cow and that of the other of the pair, the records were discarded.

The index of yield for each cow was calculated by correcting the actual yield for age according to the figures Sanders (6) obtained for Dairy Shorthorns,

and then taking the average of the number of age-corrected lactations available. In no case was the record of a cow based on less than two lactations. Any lactation extending over a greater period than 365 days or less than 245 days was discarded, as also were those where the interval between calvings exceeded fifteen months. Since the figures were taken from the private records of the breeders, and not from published sources, it was considered that it included all grades of yielders and thus eliminated a possible source of error which had existed in all previous studies. Unfortunately adequate figures for butterfat were not available. The results are shown in Table II.

Table II. *Difference in milk yield of pairs of Dairy Shorthorn cows from three herds according to their relationship classification*

	Number of pairs N	$\Sigma D^2/N$
(1) Full sisters	191	38,644
Half sisters:		
(2) By same sire with dams paternal half sisters	2,506	32,822
(3) By same sire with dams unrelated	11,903	44,155
(4) By same dam with sires unrelated	215	59,577
Cousins:		
(5) By sires which are full brothers	248	33,329
Half cousins:		
(6) By same paternal grandsire	3,627	62,856
(7) By same maternal grandsire	8,737	47,499
(8) By same paternal grandam	1,021	45,318
(9) By same maternal grandam	667	47,684
Unrelated pairs:		
(10) Random sample	7,573	78,357

Here again, with the exception of the group of half cousins by the same paternal grandsire (Class 6), the figures, where large enough, show a consistent increase as the degree of kinship widens. That the kinship group where the relationship of the pairs is based on the paternal grandsire again shows this aberration implies that this cannot be due to chance, particularly as it confirms the results obtained by less reliable methods and data. We are thus inevitably forced to the conclusion that there is some complicating factor which operates in the transmission of milk yield from one generation of cattle to another. This point is more fully discussed in a subsequent portion of this paper where the results are examined in detail.

It was suggested to us by Dr Fisher that the results obtained might be translated into correlation figures by the use of the following formula:

$$r = 1 - \frac{\text{figure for group considered}}{\text{figure for unrelated cows}}.$$

Accordingly we give, in Table III, the correlations thereby obtained in order to give an estimate of the degree of similarity existing among cows of the same group.

Caution is required in the interpretation of these figures. The figure for unrelated cows is not the best that might be used. To express the true genetic

variance the figures for unrelated cows should be based not, as has been done, on pairs of cows contained in the same data, but rather on pairs of cows of as many breeds and crosses as possible, but maintained under identical environmental conditions. There is no doubt that if it were possible to secure such data it would give a larger variance. Accordingly the correlation figure r which we have given must in every case be too small. This is, however, only of importance when it comes to a consideration of the relative forces of heredity and environment. r does not interpret the full genetic influence of the common ancestor involved, but it does provide a guide within the breed (or herd) to the relationship between an ancestor and its descendant.

Table III. *Correlations in respect of Herd A only*

	$r =$	Number of pairs
(1) Full sisters	+0·679	123
Half sisters:		
(2) By same sire with dams by same sire	+0·539	2020
(3) By same sire dams unrelated	+0·323	9543
(4) By same dam sires unrelated	+0·364	100
Cousins:		
(5) By sires which are full brothers	+0·432	248
Half cousins:		
(6) By same paternal grandsire	+0·035	2083
(7) By same maternal grandsire	+0·351	6883
(8) By same paternal grandam	+0·380	273
(9) By same maternal grandam	+0·338	476

Part II. Detailed examination of the results

In view of the fact that the method employed in this investigation is a new one it deserves some examination, particularly since it is suitable for the analysis of other genetic problems which involve quantitative inheritance, and especially so when the expression of that quantitative character is limited to one sex though transmitted by both.

In Appendix I will be found tables giving in detail the results obtained, first in respect of the Ayrshire cattle examined in the previous study, secondly in respect of each of the three herds on which this study is based, and thirdly as regards the largest of these three herds in respect of two separate sections, the low-yielding and the high-yielding animals.

The first point of interest is the number of blanks in the relationship tables. Some of the degrees of kinship are exceedingly uncommon, but this is unavoidable in a class of animals in which not only is inbreeding rare, but where the emphasis for improvement is laid upon the sire. As already pointed out, in the Ayrshire data out of 26,091 pairs of cows, 17,893 traced their connexion through a male ancestor as compared to 1858 pairs whose connexion depended on a female ancestor. In the present study 36,116 pairs were involved, and of these 2172 were connected by a female ancestor whereas 27,463 traced through a common male ancestor. (Allowance must be made for animals which fall into both male and female relationships.) Such a

difference is only to be expected in a breeding population where the females outnumber the males by somewhere about 25 to 1.

There are some interesting differences in the numbers falling into the different kinship groups in respect of the two studies. For instance there is a considerable increase in the proportion of the pairs of Dairy Shorthorn cows which are by the same sire as compared to the Ayrshire data. The number of cows on which each study is based is approximately the same, being 1518 for the Ayrshires, and 1536 in the present examination. Similar increases are found where the sires are unrelated and the dams are half sisters, either paternal or maternal. These cannot be considered to be breed differences between the Dairy Shorthorn and the Ayrshire. The Dairy Shorthorn data is confined to three large herds, and consequently a greater increase would be expected in the proportion of pairs by a common sire or a related dam as compared to the Ayrshire data where there were thirty herds, all comparatively small.

This explanation appears to be borne out by the fact that the Dairy Shorthorn data show a decrease in the number of pairs where the sires are whole brothers, and this decrease is reproduced by a marked decrease in the number of pairs where the sires are paternal half brothers. Apparently the tendency amongst these Dairy Shorthorn breeders has been to avoid using in their own herd sires which are either full brothers or which possess a common sire. Is this tendency indicative of a subconscious recognition of the fact that the influence of the paternal grandsire is of less importance than that of the other parents?

With the example of the creators of the Beef Shorthorn before them, the Dairy Shorthorn breeders have shown more inclination for inbreeding than the Ayrshire men. This in part accounts for the increase in the proportion of the pairs whose sires and whose dams have both a common sire, i.e. the group where the sires are paternal half brothers and the dams paternal half sisters.

Turning now to an examination of the figures of each of the three herds that constitute the present study, an analysis of the data for each of the three herds in respect of the relative influence of each of the grandsires is interesting. The crucial figures are as follows:

Table IV

	Herd A		Herd B		Herd C	
Kinship group	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
(5) Sires paternal half brothers. Dams unrelated	2083	56,629	1435	66,553	109	133,175
(6) Sires unrelated. Dams paternal half sisters	6883	38,086	1368	71,845	486	101,850
(9) Unrelated pairs	4414	58,665	1784	91,400	537	201,593

It will be seen that only two of these herds, A and C, show an increased variation due to the paternal grandsire, while Herd B shows a slight tendency in the opposite direction. The difference is great in respect of Herds A and C

and may be taken as significant, since in Herd A it amounts to some 30% of the total variation of the unrelated pairs, and in Herd C to 15% of the total variation. In Herd B, however, it is less than 6% of the total variation.

This anomaly in respect of Herd B may be interpreted in one of three ways. First, it may be interpreted as implying that the paternal grandsire is of equal importance with the maternal. The results of the Ayrshire data and of the other two herds would then have to be explained as due to chance. This is rather too far-reaching an assumption to make, and, if it is maintained, it can equally be said—and with three times as much likelihood of being correct—that the figure in respect of Herd B is due to chance, while the three others are the correct ones.

But, granting that the difference between Herd B and the other results is significant, it may be argued that if there is an important sex-linked gene acting, as well as considerable genetic variance, it is possible that Herd B is homozygous, or nearly so, for the sex-linked gene. This is a not unreasonable assumption.

A third interpretation may be obtained from a more detailed analysis of the figures. In this investigation, after the figures for Herd A had been analysed, and before starting on the records for Herd B, it was deemed advisable to investigate the method so far as selected groups of cows were concerned. Herd A was accordingly divided into two groups of high- and low-yielding cows respectively, according as they gave over or under 700 gallons. These groups were then treated as separate units. The detailed results are to be found with the others in the Appendix. There is reason to believe that they furnish the explanation of the figures obtained in respect of Herd B. The salient facts are as follows:

Table V

	Herd A						Herd B	
	Entire herd		High yielders		Low yielders		Entire herd	
Degree of kinship	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
(5) Sire paternal half brothers. Dams unrelated	2083	56,629	392	16,774	1171	17,237	1435	66,553
(6) Sires unrelated. Dams paternal half sisters	6883	38,086	1087	16,652	3087	17,303	1368	71,845
(9) Unrelated	4414	58,665	802	27,822	1442	21,553	1784	91,400

A study of the whole of Herd A has revealed the fact that, where the common ancestor of each pair of cows was the paternal grandsire, the variance approximated the variance found in the whole herd, and was nearly 50% greater than where the pairs of cows traced to a common maternal grandsire. But when these cows were divided into two groups of high yielders and low yielders this difference was not apparent. In each of the two divisions of the herd the variance of the two classes of half cousins is, in this respect, the same. This may be interpreted as indicating that the sex-linked factor acts as a

major modifying gene in that it always appreciably modifies the yields of the cows by depressing or increasing them to a considerable extent.

Thus the reason why no difference was obtained in respect of these two groups of half cousins in Herd B may be easily explained, especially since an examination of the data showed that the owner of Herd B had been discarding from his herd, before the completion of their second lactation, a far larger proportion of cows than had the owners of Herds A or C. Correspondence with the owner of Herd B confirmed this deduction. It is thus possible that in Herd B we are dealing with a group of cows already selected for their high yields, and that this fact has obscured the true action of a possible sex-linked gene.

These figures may be looked at also from another angle. The average lactation yield of all the cows of Herd A is 642 gallons. The dividing line was off the centre at 700 gallons. There were 388 high yielders with an average of 824 gallons, and there were 567 low yielders with an average of 575 gallons.

If the herd were divided equally, then in the group of unrelated cows the individual cows should be found equally on both sides of the dividing line. This being the case, a high-yielding cow should have an equal chance of being compared either with another high-yielding cow or with a low-yielding cow. If the low yielders are excluded, the high-yielding cow can only be compared with another high-yielding cow, and the number of pairs possible for comparison is reduced by half. If 50% of all the cows are high yielders, theoretically the possible number of pairs of high yielders is only 25% of the total number of pairs of unrelated cows in the whole herd. Similarly, as regards low yielders, the possible number of pairs is only 25% of the total number of pairs of unrelated cows in the whole herd.

Then (without allowing for the fact that the herd has not been quite equally divided), in respect of the unrelated pairs, the total number of pairs of each of the two subdivisions of the herd should be approximately half the total number of pairs for the whole herd. This is the case, the total number of pairs of the two subdivisions amounting to 51% of the whole. That is to say, 49% of the pairs of the whole herd have been lost by the subdivisions: such pairs are referred to as *divided pairs*.

Genetic factors increase the similarity in yield between related pairs; therefore, with related pairs, the chance increases that a high yielder will more likely be paired with a high yielder and that low yielders will stand a better chance of pairing with low yielders. Accordingly, the number of *divided pairs* should be less than 50%. This appears to be approximately true, except in one instance. Where the numbers are considered significant the percentage of *divided pairs* in respect of each relationship group is as shown in Table VI.

The significant point is the fact that where half cousins are related by a common paternal grandsire 75% of the pairs fall into one or other of the two groups. (This figure is perhaps unduly high owing to the fact that the herd has not been equally divided, but that this is not a major objection is seen

Table VI. *Herd A. Division of pairs of high and low yielders*

	Number of pairs in whole herd	Percentage divided pairs
(2) Sires same. Dams paternal half sisters	2020	48
(3) Sires same. Dams unrelated	9543	45
(6) Half cousins. Same paternal grandsire	2083	25
(7) Half cousins. Same maternal grandsire	6883	39
(9) Unrelated pairs	4414	49

from the comparatively even distribution of the other groups.) But, *within each subdivision* of group 6 the variability is the same as that to be expected if the inheritance were autosomal (Table V). The variability found for this group in the whole herd is not the sum of the variabilities found in respect of 75% of the total number of pairs which fall into the high- and low-yielding subdivisions (i.e. 56,629 is not the sum of 16,774 + 17,237). Their variabilities combined are roughly the same as the variability of the group of half cousins tracing to a common maternal grandsire (38,086). The fact that *in the whole herd* the variability of half cousins tracing to a common paternal grandsire is so much greater implies that the cause for great variability must lie in the 25% of divided pairs.

Hence this variability must be of a very high order indeed. The individuals which comprise the divided pairs instead of each having yields lying, the one just below 700 gallons and the other just above that figure (as must be the case in the other relationship groups), must have yields extremely far apart, and in all probability beyond the average of each of the subdivisions. The average lactation yield of the high-yielding cows is 824 gallons, while that of the low-yielding cows is 575, the difference being 249 gallons. If, in the entire herd, the variability of this group of half cousins tracing to a common paternal grandsire is due to a sex-linked factor, and if this sex-linked factor is not expressed in respect of 75% of the pairs examined, it follows that the extreme variability of the 25% is an indication of the variability due to the sex-linked gene, which consequently appears to have an effect of some 250 gallons in a herd of cows whose average yield is 650 gallons. In other words, it may be postulated that there is a sex-linked gene which conditions the total yield of milk, and which, in this case, affects about one-third of the total yield. The reason why this "scatter" is not seen in the random sample of the unrelated pairs is that the unrelated pairs, when subdivided, do not have the autosomal inheritance to link the pairs together, and consequently to reduce the percentage of divided pairs.

As regards the other relationship groups, all of which approach 50% of divided pairs, the fact that the index of variation is not as different (although, of course, there is a relatively large difference) as in the relationship group tracing to a common paternal grandsire, indicates that the yields of the

divided pairs of the other relationship groups are closer to each other and nearer to the average. This may be expressed diagrammatically as follows:

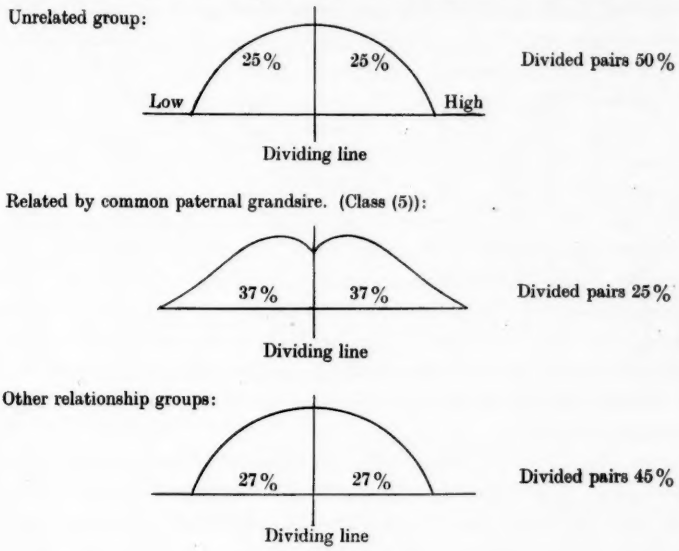


Fig. 1

In effect, the argument is this: If only a sex-linked factor operates, a random population should be divided into two classes. But if autosomal factors also operate (as they most certainly do in this case), the sex-linked factor will not segregate the population into two distinct classes. The autosomal factors have already given rise to great variation, which the sex-linked factor will only accentuate without dividing the population. The related groups, other than that possessing a common paternal grandsire, will show a reduced variation in the yields of the pairs owing to the fact that both the autosomal and the sex-linked inheritance reduce this variation. Environment is bound to account for some variation. Since a large number of pairs will have their yields near the average, it is reasonable to assume that the distribution of the pairs in respect to the dividing line should approximate the 50% of the random pairs. This is what happens.

With the pairs linked by a common paternal grandsire, the sex-linked inheritance is of no influence, and sex-linked genes operate more or less at random as they do in the general population. The fact, however, that these pairs are partly linked by autosomal inheritance reduces their variability somewhat. But the distribution of the sex-linked gene roughly divides this group of pairs into two classes, with the result that only 25% of all the pairs in this group are separated by the dividing line.

In view of the fact that in this relationship group the 25% of divided pairs must show a very high degree of variability, it is reasonable to assume that,

for this 25%, the yields of each individual must fall, in the case of the low yielders, near or below the average of the low yielders, and, in the case of the high yielders, near or above the average of the high yielders. The difference in the averages of the low and high yielders is approximately 250 gallons, and this may be taken as about the difference in the yields of the divided pairs. As it has been argued that the differences in the divided pairs are primarily due to the random effect of the sex-linked gene, it is possible to make the deduction that the effect of the sex-linked gene is of the order of 250 gallons in a population of cows averaging about 650 gallons.

The next step is to determine the manner of the inheritance of the sex-linked gene, dominant, recessive, or intermediate. Were it recessive it might be assumed that the population could soon become homozygous for it. But the fixing of a recessive that is both sex-linked and sex-limited is not so easy as the fixing of a simple recessive. Nor can it be said that breeders have intensively selected for such a recessive, for they have other characters for which they must select their stock, and the effect of sex-linkage may be masked by autosomal inheritance.

In another paper(7) dealing with the variance in the progeny of different bulls whose daughters are out of the same population of cows and were in-milk at the same time, it has been shown that such bulls may leave their daughters with very different degrees of variance, although the average yield of the daughters of the bulls is approximately the same. The paper referred to was based on the data of the three herds used in the present study. This fact points to a certain degree of dominance either in the autosomal or the sex-linked genes, or both.

In order further to examine this point the variance of the progeny of the different bulls was examined in relation to the average yield of the progeny. Only when a bull had at least six daughters meeting these conditions were his progeny considered. As only nine such bulls were found in Herd C, this herd was eliminated from the calculations. The results for the other two herds are as follows:

Table VII. *Variance in the yields of daughters of different bulls correlated with the average yield of the daughters*

	r	P.E.	N	Average yield of daughters gallons
Herd A	+0·33	±0·09	43	660
Herd B	+0·43	±0·11	24	858

These figures are not conclusive but indicate that, as the yield increases, so does the variance of the daughters. If this is so it is an argument against dominance in any high degree.

In order to clear up the point that the standard deviation, measured in gallons, may not be comparable at all levels of yields, it was decided to test the coefficient of variation in correlation with the average yields. For the data

in Herd A it was found that $r = -0{\cdot}17 \pm 0{\cdot}08$. This means little, and does not confirm the previous result. This method is probably an over-correction.

Another test could be made by ascertaining if the regression of the standard deviation on yield was higher among tested bulls than it was between different herds. Accordingly the following calculations were made:

Table VIII. *Showing coefficients of correlation and regression among daughters of selected bulls as compared to the cows in the two divisions of Herd A*

Data	Coefficient of correlation S.D. and yield	Coefficient of regression S.D. on yield	Average yield gallons
Herd A selected	$+0{\cdot}33 \pm 0{\cdot}09$	$x_1 = +0{\cdot}2080\, x_2$	660
Herd B selected	$+0{\cdot}42 \pm 0{\cdot}08$	$x_1 = +0{\cdot}2467\, x_2$	858
Herd A high yielders	$+0{\cdot}89 \pm 0{\cdot}03$	$x_1 = +0{\cdot}9215\, x_2$	787
Herd A low yielders	$-0{\cdot}42 \pm 0{\cdot}08$	$x_1 = +0{\cdot}3540\, x_2$	570

These figures tend to confirm the previous deduction that the genetic factors affecting high yield do not act in an exclusively dominant manner.

PART III. REVIEW AND CONCLUSIONS

In 1928 the present writer put forward the suggestion that the inheritance of total yield of milk might be in part conditioned by the action of sex-linked genes. This was founded on a study of the Jersey breed in England(9) and an examination of the results obtained by Gowen(1) for the Holstein breed in America. In Gowen's work it was found that the correlation to the paternal grandsire was considerably less than that to the maternal grandsire. This finding has since been confirmed by the work of Buchanan Smith *et al.*(4) with the Ayrshire breed in Scotland, and by Madsen(8) with the Red Danish breed. In the present study the figures of Herd A were analysed by this method with the following results, which confirm the previous work:

Table IX. *Correlations to paternal and maternal grandsires*

Correlation	
Sires' daughters to Sires' sons' daughters	$r = -0{\cdot}20 \pm 0{\cdot}16$
Sires' daughters to Sires' daughters' daughters	$r = +0{\cdot}43 \pm 0{\cdot}13$

These methods were based on indirect correlation and so were not altogether satisfactory. Accordingly, on the advice of Dr R. A. Fisher, the method of the squared difference employed in the present work was used in an examination of data from thirty herds of Ayrshire cows. As regards butterfat yield (in lb.) no difference was found between pairs of cows according as to whether they traced to a common paternal or maternal grandsire: but as regards milk yield the difference was significant. This difference, as regards milk yield, is confirmed in the present work.

The original observation of the possible existence of a sex-linked factor was made more than nine years ago, and in the interval no direct evidence has been led against this observation despite the fact that several workers

are engaged upon the problem. Without leading evidence in support, Gowen (15) reports that sex-linked factors do not operate. Fohrman & Graves (16), working with American Ayrshire cows, report finding no evidence of sex-linkage, but their inquiry was not so directed as to enable them to determine this point. Copeland (17), who has obtained figures in harmony with the sex-linked hypothesis, is inclined to attribute the differences to environment and methods of selection. Leroy (18), in a brief review of the subject, states that neither fat nor milk yield is sex-linked. Taufer (19) is of opinion that sex-linkage may exist, and quotes the work of Bogdanor to this effect in the case of the inheritance of fat.

The original criticism concerning the method of the correlations has been met, and subsequent results, by the improved method, have confirmed the earlier deductions.

Another criticism was in the fact that it was unjust to compare yields of cows of one generation with those of another. These have also been met by the present method which only compares the yields of cows milking almost simultaneously with each other.

A third criticism which was valid in respect of all the earlier work, including the study of Buchanan Smith & Robison (5) by the squared difference method, was that the granddaughters of paternal grandsires were more likely to be distributed throughout different herds than the granddaughters of maternal grandsires, and therefore their yields would be more subject to environmental influences. This last criticism has been met in the present paper by confining the yields compared to cows in one herd only.

Thus all the major objections have been met and there is still evidence of the fact that the paternal grandsire has less influence on the yields of his granddaughters than has the maternal grandsire. And no evidence in the contrary direction has been obtained.

The practical implications of this result are that, in the selection of a bull for a herd, undue emphasis should not be placed on his sire. It is to the good that he should be a proven good sire, since he is then bound to have some of the desired autosomal genes. But as regards the desired sex-linked gene, this can only be obtained by the bull from his dam, and therefore the yield of the dam of a bull is of prime importance. As long ago as 1925 Gowen (10) showed that the sons of proven good sires leave daughters which are, on the average, no better and no worse than the average of the breed. This has since been confirmed by other studies.

The other implication refers to inbreeding. If sex-linkage is important, then for inbreeding to be effective it cannot work by the concentration of the blood of a famous sire through his sons. In this connexion it is interesting, and perhaps significant, to note that inbreeding has not been employed successfully as a means for the creation or improvement of the dairy breeds, although it has been of the greatest use to the breeders of beef cattle. This is particularly marked in the case of the Shorthorn breed, where the Dairy Shorthorn men

have had every temptation to employ inbreeding but have not done so. One is almost drawn to the deduction that some Dairy Shorthorn breeders must have tried inbreeding, but without success. Furthermore, examinations which have been made of the coefficients of inbreeding of high-yielding dairy cows as compared to the average of the breed usually show the high yielders to be less inbred. Fowler(11) has shown this for the Ayrshire, and Buchanan Smith for the English Jersey(9) and for the Shorthorn (unpublished).

In previous papers(12, 13, 14) the present author has reviewed the literature on the subject of the inheritance of milk yield generally, and has discussed in detail the question of sex-linkage. For the convenience of readers of this paper, previous results, as they bear on the question of sex-linkage, are to be found tabulated in Appendix II.

Finally, it is the author's opinion that, for the solution of problems concerning the improvement of our slow-breeding farm animals, the statistical method based on large numbers of animals nurtured under different environments can never be more than a reconnaissance of the general problem. There can be no question that milking capacity is dependent for its transmission on a large number of genes interacting with each other. Some of these will almost certainly be borne on the sex chromosomes. The point is whether these sex-linked genes are of major importance. To this it cannot be said that the present studies have given a conclusive answer. The most that has been accomplished so far is an indication that sex-linked genes may have a certain effect, and that there is certainly no substantial evidence pointing in the other direction. Even if it should be later shown that a sex-linked gene does not operate, the curious aberration of the figures of yields of cows related by a common paternal grandsire will have to be explained. The one thing that can be definitely concluded from these studies is that an unexplained factor exists. If this unexplained factor is not genetic but environmental, it requires to be discovered, since it most certainly influences the standard of selection by which the breeder of dairy cattle operates. The problem, therefore, calls for a solution if the yields of dairy cattle are to be improved.

Until the physiologist links up with the geneticist so that the immediate and not the ultimate action of the different genes that affect milking capacity can be determined, it is unlikely that any definite information will be obtained as to the mode of inheritance of specific genes. Present investigations can only lead us to an uncertain determination that a certain gene affects, say, total yield of milk. We really want to know *how* that gene affects the yield of milk, e.g. whether it is through a full functioning of the pituitary gland. Then it will be possible to discriminate one gene from another and to trace the mode of its inheritance.

In the meantime the immediate information we require is whether sex-linked genes do or do not play a major part in the transmission of milk yield in order that we may modify our method of selection accordingly. It seems that the only way by which this information can be obtained is by a planned

experiment in a herd where environmental effect has been reduced to a minimum. Further statistical study of the type cited in this paper cannot give conclusive results which will be sufficient for practical work. We must know whether sex-linkage is of practical importance in this problem. Accordingly the present author does not propose to make any further investigations of this nature, for the magnitude of the task of analysing such data is not commensurate with the degree of accuracy of the results obtained.

Summary

The paper embodies an examination of the possibility of sex-linked genes affecting the transmission of milk yield. To reduce environmental effect the study was restricted to three herds of the English Dairy Shorthorn breed, and each herd was considered as a unit. Fisher's Squared Difference method was used.

The lumped results confirm previous work to the effect that the paternal grandsire has a lesser effect than the maternal grandsire. In this respect the figures for one herd did not agree with the other two.

The largest herd (which showed this difference) was divided into two sections, high and low yielders. These sections did not show any difference in the relative effect of the two grandsires. The implications of this are discussed, and it is tentatively suggested that if sex-linkage operates it has a considerable effect of some 250 gallons on an average yield of 600 gallons.

The whole subject is reviewed, and the author concludes that the accuracy of the conclusions which may be drawn from statistical studies of this type are not commensurate with the labour involved. As a primary reconnaissance such studies have definite value, but planned experimentation is now required in order to resolve the problem.

Acknowledgements

The author desires to express his gratitude to Dr O. J. Robison for assistance in calculation and tabulation of the figures: to Dr R. A. Fisher for many suggestions, to Dr H. P. Donald, and to Prof. F. A. E. Crew.

Table X. *Ayrshire cows* (*Buchanan Smith & Robison*, 1931). *Differences in the butterfat yield of pairs of cows according to their relationship classification*

	Relationship of sires									
	Same		Whole brother (same sire, same dam)		Paternal half brother (common sire)		Maternal half brother (common dam)		Unrelated	
Relationship of dams:	(I) N	(II) $\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	200	4150	—	—	7	5988	—	—	158	4358
Whole sister (same sire, same dam)	41	3285	—	—	—	—	—	—	29	3270
Paternal half sister (common sire)	2027	4545	3	4292	29	4812	2	1700	2303	6576
Maternal half sister (common dam)	69	4028	—	—	1	361	—	—	185	5389
Unrelated	6361	4973	415	4742	6435*	6435*	1166	6036	6660	7950

* These figures are correct. The coincidence is remarkable.

(I) N = Total number of pairs in each classification group.
(II) $\Sigma D^2/N$ = Mean of summation of squared differences between the yields of pairs of cows in each group.

Table XI. *Ayrshire cows* (*Buchanan Smith & Robison*, 1931). *Differences in the milk yield of pairs of cows according to their relationship classification*

	Relationship of sires									
	Same		Whole brother (same sire, same dam)		Paternal half brother (common sire)		Maternal half brother (common dam)		Unrelated	
Relationship of dams:	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	200	24,822	—	—	7	34,099	—	—	158	32,837
Whole sister (same sire, same dam)	41	12,260	—	—	—	—	—	—	29	9,699
Paternal half sister (common sire)	2027	27,821	3	1,292	29	12,264	2	9,425	2303	24,218
Maternal half sister (common dam)	69	27,176	—	—	1	15,129	—	—	185	33,191
Unrelated	6361	35,698	415	55,920	6435	49,931	1166	44,128	6660	45,579

Total number of pairs of cows = 26,091.
Average lactation yield in gallons = 910. S.D. = 159·5.
Number of cows = 1,518.

Table XII. *Summary of data on Dairy Shorthorns—Milk yield*

Herd	Number of cows	Number of pairs	Average lactation yield	S.D.	S.E.
Herd A complete	955	26,315	642	145·0	± 3·38
Herd A high yielding	388	4,106	824	103·5	± 2·55
Herd A low yielding	567	10,756	575	187·5	± 2·58
Herd B	369	7,523	830	196·0	± 7·21
Herd C	212	2,278	721	238·0	±11·7
Combined herds	1536	36,116	698	134·5	± 2·44

Table XIII. *Dairy Shorthorns—Combined herds. Differences in milk yield of pairs of cows according to their relationship classification*

	Same		Whole brother		Paternal half brother		Maternal half brother		Not brother	
	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	191	38,644	6	27,225	21	69,351	—	—	215	59,577
Whole sister	15	78,584	—	—	—	—	—	—	13	37,761
Paternal half sister	2,506	32,882	11	50,759	177	41,521	5	19,506	8737	47,499
Maternal half sister	16	29,731	—	—	2	165,698	—	—	667	47,684
Unrelated	11,903	44,155	248	33,329	3627	62,856	1021	45,318	6735*	78,732

Total number of pairs of cows = 36,116.
Average lactation yield in gallons = 698. S.D. = 134·5.
Number of cows = 1,536

* Another random sample for this group gave 7573 pairs and $\Sigma D^2/N$ 78,357.

Table XIV. *Dairy Shorthorns—Herd A*

	Same		Whole brother		Paternal half brother		Maternal half brother		Not brother	
	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	123	18,827	6	27,255	1	14,884	—	—	100	37,337
Whole sister	9	50,926	—	—	—	—	—	—	—	—
Paternal half sister	2020	27,057	4	31,919	118	32,769	—	—	6883	38,086
Maternal half sister	12	20,840	—	—	2	165,698	—	—	476	38,864
Not sister	9543	39,705	248	33,329	2083	56,629	273	36,348	4414	58,665

Total number of pairs of cows = 26,315.
Average lactation yield in gallons = 642. S.D. = 145·0.
Number of cows = 955.

Table XV. *Dairy Shorthorns—Herd B*

	Same		Whole brother		Paternal half brother		Maternal half brother		Not brother	
	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	34	39,667	—	—	20	72,081	—	—	104	82,616
Whole sister	2	25,065	—	—	—	—	—	—	13	37,761
Paternal half sister	204	51,986	7	61,526	59	59,023	5	61,526	1368	71,845
Maternal half sister	4	56,402	—	—	—	—	—	—	168	79,550
Not sister	1568	53,007	—	—	1435	66,553	748	48,592	1784	91,400

Total number of pairs of cows = 7523.
Average lactation yield in gallons = 830. S.D. = 196·0
Total number of cows = 369.

Table XVI. *Dairy Shorthorns—Herd C*

	Same		Whole brother		Paternal half brother		Maternal half brother		Not brother	
	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	34	109,311	—	—	—	—	—	—	11	43,932
Whole sister	4	167,578	—	—	—	—	—	—	—	—
Paternal half sister	282	68,794	—	—	—	—	—	—	486	101,850
Maternal half sister	—	—	—	—	—	—	—	—	23	38,848
Not sister	792	80,254	—	—	109	133,175	—	—	537	201,593

Total number of pairs of cows =2278.
Average lactation yield in gallons = 721. S.D. =238·0.
Number of cows = 212.

Table XVII. *Dairy Shorthorns—Herd A—High-yielding cows*

(700 gallons and over)

	Same		Whole brother		Paternal half brother		Maternal half brother		Not brother	
	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	31	12,211	—	—	1	13,225	—	—	45	16,156
Whole sister	—	—	—	—	—	—	—	—	—	—
Paternal half sister	293	11,482	8	20,679	14	23,128	—	—	1087	16,652
Maternal half sister	6	59,549	—	—	6	8,186	—	—	39	10,527
Not sister	1354	13,144	28	14,492	392	16,774	—	—	802	27,822

Total number of pairs of cows =4106.
Average lactation yield in gallons = 824. S.D. =103·5.
Total number of cows = 388.

Table XVIII. *Dairy Shorthorns—Herd A—Low-yielding cows*

(Under 700 gallons)

	Same		Whole brother		Paternal half brother		Maternal half brother		Not brother	
	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$	N	$\Sigma D^2/N$
Same	63	12,391	1	14,161	5	16,239	—	—	69	17,008
Whole sister	6	31,316	—	—	—	—	—	—	8	14,472
Paternal half sister	764	14,693	2	42,027	38	29,579	—	—	3087	17,303
Maternal half sister	8	30,774	—	—	—	—	—	—	99	16,059
Not sister	3894	13,748	68	26,105	1171	17,237	31	13,275	1442	21,553

Total number of pairs of cows =10,756.
Average lactation yield in gallons = 575. S.D. =87·5.
Total number of cows = 567.

APPENDIX II

Correlations, etc., showing the relative effect of the paternal and maternal grandsires upon the milking capacity of their granddaughters

Gowen (1924): American Holstein

	Milk yield	Butterfat %
Granddaughter to Paternal grandsire	+0·070 ±0·014	+0·176 ±0·014
Granddaughter to Maternal grandsire	+0·244 ±0·016	+0·224 ±0·016
Half cousins by Paternal grandsire	+0·005 ±0·029	+0·119 ±0·029
Half cousins by Maternal grandsire	+0·206 ±0·020	+0·216 ±0·020

Gowen (1934): American Jersey

	Milk yield
Pairs by common Paternal grandsire	+0·02 (489,526 pairs)
Pairs by common Maternal grandsire	+0·12 (64,150 pairs)

Buchanan Smith, Scott & Fowler (1930): Scottish Ayrshire

	Milk yield
Granddaughter to Paternal grandsire	+0·259 ±0·016
Granddaughter to Maternal grandsire	+0·478 ±0·013
Sires' daughters to Sires' sons' daughters (i.e. Paternal grandsire link)	+0·253 ±0·070
Sires' daughters to Sires' daughters' daughters (i.e. Maternal grandsire link)	+0·322 ±0·041

Smith & Robison (1931): Scottish Ayrshire

(Correlation based on Squared Difference method)

	Milk yield	Fat yield
Granddaughters by same Paternal grandsire	−0·1	+0·19
Granddaughters by same Maternal grandsire	+0·469	+0·173

Madsen (1932): Red Danish

	Milk yield	Butterfat yield
Bulls to Paternal grandams	+0·026	+0·061
Bulls to Maternal grandams	+0·112	+0·134

Standard error varies from +0·03 to ±0·04.

Note. This study is of the genotype of *bulls* and therefore the crucial correlations are to the grandams, thus showing the relative importance of the sire and dam in the transmission of milking capacity to the bull.

Copeland (1934): American Jersey

Paternal grandsires' daughters to Grandsons' daughters: Butterfat yield +0·250 ±0·036.
Maternal grandsires' daughters to Grandsons' daughters: Butterfat yield +0·427 ±0·036

Transmitting ability of three Jersey bulls in lb. butterfat

	Daughters		Paternal grandsons		Maternal grandsons	
Grandsire	No.	Average yield	No.	Average yield of their daughters	No.	Average yield of their daughters
Pgg	119	693·88	12	580·86	10	619·04
F. P.	62	728·08	5	557·52	5	604·22
H. F. T.	72	637·34	14	581·03	22	600·57

Copeland attributes the higher average yield of the maternal granddaughters to the fact that there is a tendency for the daughters of maternal grandsires to be a more highly selected group than the sons of paternal grandsires. He notes that the dams of tested sires are a highly selected group.

Average lactation yield in gallons = 575. S.D. = 87·5.
Total number of cows = 567.

Buchanan Smith (1937):

English Dairy Shorthorns (this study)

(Correlation based on Squared Difference method)

	Milk yield
Granddaughters by same Paternal grandsire	+0·198
Granddaughters by same Maternal grandsire	+0·394
Herd A only:	
Granddaughters by same Paternal grandsire	+0·035
Granddaughters by same Maternal grandsire	+0·351
By indirect correlation, Herd A:	
Sires' daughters to Sires' sons' daughters (i.e. Paternal grandsire link)	−0·20 ±0·16
Sires' daughters to Sires' daughters' daughters (i.e. Maternal grandsire link)	+0·43 ±0·13

REFERENCES

(1) Gowen, J. W. (1924). *Milk Secretion.* Williams and Wilkins, Baltimore.

(2) Patow, C. v. (1930). Weitere Studien über die Vererbung der Milchleistung beim Rinde. *Z. Zucht.* B, **17**, 3–159.

(3) Gifford, W. & Turner, C. W. (1928). The Mode of Inheritance of Yearly Butter-Fat Production. An Analysis of the Progeny Performance of Ayrshire Sires and Dams. *Res. Bull. Mo. agric. Exp. Sta.* **120**, 1–52.

(4) Smith, A. D. Buchanan, Scott, R. J. & Fowler, A. B. (1930). The Inheritance of Milk Yield in Ayrshire Cows. *J. Dairy Res.* **1**, 174–9.

(5) Smith, A. D. Buchanan & Robison, O. J. (1931). The Inheritance of Milk Yield. IX. *Int. Dairy Congr.* Paper No. 4, 127–40.

(6) Sanders, H. G. (1927). *Variations in Milk Yields caused by Season of the Year, Service, Age, and Dry Period, and their Elimination.* Cambridge University Press.

(7) Smith, A. D. Buchanan & Robison, O. J. (1937). Variations in the Milk Yields of the Daughters of Different Bulls. IX. *Int. Dairy Congr.*, **1**, 60–3.

(8) Madsen, K. (1932). Inheritance of Milking Capacity. *Nature, Lond.*, **129**, 165–6.

(9) Smith, A. D. Buchanan (1928). Inbreeding in Jersey Cattle. *Rep. Brit. Ass.* **22**, 649–55.

(10) Gowen, J. W. (1925). Studies in Milk Secretion, XVI. Progeny Performance of Guernsey Sires' Sons. *Bull. Maine agric. Exp. Sta.* **327**, 197–252.

(11) Fowler, A. B. (1932). The Ayrshire Breed of Cattle: A Genetic Study. *J. Dairy Res.* **4**, 11–27.

(12) Smith, A. D. Buchanan & Robison, O. J. (1933). The Genetics of Cattle. *Bibliogr. genet.* **10**, 104 pp.

(13) Smith, A. D. Buchanan (1935). Physiology of Dairy Cattle—Genetics. *J. Dairy Res.* **6**, 272–81.

(14) —— (1937). Physiology of Dairy Cattle—Genetics. *J. Dairy Res.* **8**, 131–42.

(15) Gowen, J. W. (1934). Influence of Inheritance and Environment on the Milk Production and Butterfat Percentage of Jersey Cattle. *J. agric. Res.* **49**, 433–65.

(16) Fohrman, M. H. & Graves, R. R. (1933). Analysis of the Advanced Registry Records of 611 Daughters of 51 Ayrshire Sires. *Tech. Bull. U.S. Dep. Agric.* No. 349, 59 pp.

(17) Copeland, L. (1934). Pedigree Analysis as a Basis of Selecting Bull Calves. *J. Dairy Sci.* **17**, 93–102.

(18) Leroy, A. M. (1936). Paper presented at Congress of Animal Breeding, Paris, December, 1936. Abst. *Rec. Méd. vét.* **113**, 232–9, 1937.

(19) Taufer, J. (1932). Příspěvek k otázce pohlavní limitace dědičnosti dojivosti a mléčného tuku u skotu. *Věstn. čsl. Akad. Zeměd.* **8**, 578–81.

(*MS. received for publication* 25 *September* 1937)

637:576.8

BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION B. BACTERIOLOGY AND MYCOLOGY APPLIED TO DAIRYING

I. Milk production and control.
(*a*) Technique.
II. Micro-organisms in milk and milk products.
(*a*) Coli-aerogenes group.
(*b*) Milk taints and other faults.
(*c*) Bacteriophage.
III. Lactic acid and allied fermentations.
(*a*) Bacterial metabolism.
(*b*) Lactic acid bacteria.
(*c*) Fermented milks.
(*d*) Starters.
(*e*) Cheese.
(*f*) Butter.
IV. Pasteurization and other processes.

I. MILK PRODUCTION AND CONTROL

(*a*) Technique

Since the publication of the last review the most outstanding contribution to the bacteriology of milk as regards the assessment of hygienic quality has come from Wilson *et al.* (1). These authors, after several years' work, have produced the most comprehensive and valuable series of observations which have yet appeared in this field. It is not possible in the space at our disposal to review this book at all adequately, but it should be carefully studied by all who are interested in milk examination.

Part I. Section A deals with the plate count of milk and begins with the collection of samples, the effect of cooling, and the conditions of holding before examinations are made. The use and errors of pipettes and specifications for standard use together with the effects of diluents are then dealt with. A particularly valuable section on the method of making dilutions and upon the choice of medium follows. A comparison of milk agar, the medium of choice, with the standard medium of the A.P.H.A. is also given. The effects of volume of medium per plate, the method of pouring and mixing and of the temperature of incubation are dissected and fully recorded. The important questions of the influence on the final count of methods of counting are examined and suitable counting devices described. The error of counting itself, to which attention was directed in a previous review, and the error due to overcrowding and related causes have been determined. Finally, in this section the effect of the error accompanying the number of plates used for an estimate of the plate count on the error and the total error of the plate count are dealt with in detail. Resulting from this work a complete account of a method of performing the plate count from sampling the milk to reporting the result is given. The milk-agar medium used by the authors and the method they recommend has with few altera-

tions now been officially adopted in England and appears in Memo. 139/Foods, Ministry of Health.

Section B deals in an equally comprehensive way with the coliform count of milk, and much work on the factors affecting the quantitative estimation of these organisms is recorded. The identification of the individual components of the coliform flora has also received attention, and the value of the Eijkman test at 44° C. for the identification of true faecal types has been demonstrated. The grave errors attending the expression of the numbers present in any quantitative way when small numbers of tubes are used for a determination are revealed.

Section C is wholly concerned with the methylene-blue reductase test beginning with a review of the literature on the reducing systems found in milk. The old method of carrying out the methylene-blue reductase test was inapplicable to milk containing few micro-organisms because of the rising of the fat to the surface during the performance of the test. The cream itself contains reducing substances, and the bacteria which are swept up with it are removed from the sphere of reaction. To overcome this difficulty the authors introduced the modification of closing $6 \times \frac{5}{8}$ in. tubes containing 10 ml. of milk and the requisite amount of dye with a rubber stopper and inverting half-hourly. This resulted in material improvement in the value of the results. This is the most practical outcome of the work recorded in this section which, however, contains much biochemical work of fundamental interest. The activity of leucocytes in reducing methylene blue is (perhaps surprisingly in view of the work of previous authors) shown to be small under aerobic conditions. Earlier observations that dead organisms do not contribute to methylene-blue reduction appear to be confirmed. Investigations with raw and pasteurized milk on the flora and the numbers of the various types at the time of reduction of methylene blue when the test was made at 17·5, 30, 37, and 55° C. were made. The reducing activity of a number of types including coliforms streptococci, staphylococci, micrococci and *Achromobacterium* were determined. The staphylococci were amongst the most active reducers. A consideration of the reducing systems in milk in which bacterial growth was active led to the conclusion that the reduction test may be regarded as a measure of the metabolic activities of bacteria present in the flora. In this way, of course, it differs fundamentally from the plate count. This conclusion has led the authors to urge the superiority of the reductase test because the plate count, even if it were accurate, cannot measure the "potential" due to the accumulation of "events" even before bacterial division has actually taken place. The methylene-blue test therefore is claimed to be a better measure of probable keeping quality than the plate count. Comparison of the modified with the old methylene-blue test showed that in general, especially with raw milk, the reduction times were considerably shorter with the former. As a result of experiments on the effect of temperature of incubation on reduction time the authors concluded that for practical purposes a temperature between 37 and 38° C. is to be preferred to one between 36 and 37° C.; in other words, a slight rise over 37° C. is to be preferred to a slight fall. On determining the total error of performing the modified methylene-blue test it was found that the coefficient of variation was as low as 1·12%, a figure of the order of 20 times less than that of the plate count.

Section D gives an account of the examination of a number of tests used or advocated for milk grading. The sediment test, the leucocyte count, the Breed Smear Method, titratable acidity, the Brom Thymol Blue Test, the laboratory pasteurization test and the Frost Little Plate Method were all described. In Section E, which is concerned with the interpretation of the tests, all these methods were used to test milk from farms and pasteurized milk, and the relationships between them were shown as correlation coefficients.

It is finally concluded that the plate count is liable to such serious errors that its use for *grading* ordinary raw milk should be abandoned. Mattick *et al.*(2), in reporting the results, examined statistically, of a large and long continued co-operative experiment involving ten laboratories during the years 1928–32, found that the errors from laboratory to laboratory were so large, owing to the hazards of transport of the samples, that the practice of comparing counts made on the same milk in different laboratories should be discontinued unless rigid precautions to maintain the same conditions are taken. Even when different individual samples are examined by the same technique by two workers it is necessary to allow a wide margin before differences can be asserted to be significant.

The condemnation of the plate count for grading purposes will be a bitter pill for many to swallow, but the honest worker will not attempt to sustain his personal preference in the face of statistical evidence. This is not to say that the plate count has ceased altogether to be of value in milk work. For first class raw milk and, so far as can be seen at present, for pasteurized milk and for advisory purposes it retains its value in the hands of those who understand its limitations.

The search for the best medium and technique, presumably those yielding the greatest number of colonies, continues, as witness the work of Bowers & Hucker(3). Many of these media tend to complication of ingredients—an undesirable feature of any standard work. If the writers may express an opinion it is that in the simple milk agar described by Hiscox *et al.*(4) with few modifications in preparation, or in that of Memo. 139/Foods, we have a medium which is adequate for its purpose. Similar controversy rages around the question of the best temperature of incubation to use. Yale & Pederson(5) insist upon 32° C. for 2 days as superior to 37° C. Wilson *et al.*(1), criticizing the adoption of 32° C., maintain a preference for 37° C. From the public health point of view 37° C. favouring, as in water analysis, the animal parasitic type of organism is preferable, but it is at least arguable that so little information of public health value is afforded by the ordinary plate count that if it could be shown that 32° C. or other temperature (Demeter(6)) gave more information on the cleanliness of production and proper handling it should be used, when the "commercial" quality of milk is in question.

The modified methylene-blue test which is now adopted as the standard method for the examination of accredited and tuberculin-tested milk in England (Memo. 139/Foods) has not escaped criticism by some workers, although it has been approved by others(7). Malcolm & Leitch(8) claim that in winter milk may contain comparatively large numbers of bacteria and still give a long reduction time. If the presence of these organisms is held to indicate bad methods of production and the methylene-blue test is being used as a means of assessing them, it may be that some modification of procedure is called for if the results of Malcolm & Leitch are confirmed in other areas. It should, however, be remembered that the methylene-blue test is a measure of "activity", and in spite of the presence of numbers of bacteria the keeping quality may still be good. This would possibly mean a return to the plate count as a means of assessment of conditions of production and handling at the farm, on the excellence of which bonus payments are made. We shall await with interest the repetition by others of the work of Malcolm & Leitch.

Owing to the alleged difficulty of purifying the chloride, Thornton *et al.*(9) propose the substitution of the thiocyanate of methylene-blue which reduces over the same range and behaves similarly at about equal concentrations.

II. MICRO-ORGANISMS IN MILK AND MILK PRODUCTS

(*a*) *Coli-aereogenes* group

The organisms of this group are amongst the most important of those with which the dairy industry is concerned, and they, therefore, receive special attention in the literature relating to dairying. Much of the work done on this group deals with the detection and identification of its members in water and milk supplies, a matter of considerable importance to the milk industry. But there are other lines of research which, though at present they apparently have little direct bearing on problems of milk production and utilization, are of value in throwing light on the relationship and development of the various species. Variations are frequent among members of this group, and a closer study of dissociation and the rise of variants may eventually help to solve the problem of classification within the group. Amongst work of this nature may be mentioned Dombrowsky's (1) microscopical observations of the origin and development of various cell forms in single-cell cultures of *Bact. coli commune*, Rennebaum's (2) description of three rough variants of *Bact. coli* which differed markedly from the parent strain in their fermentative capacity, and Torrey & Montu's (3) studies (including serological tests) of R and S variants, also O group and mucoid derivatives of *Bact. coli*. Bruschettini (4) attempted to differentiate between *Bact. coli* of human and animal origin, but found biochemical and even serological tests unsatisfactory. A strain of *Bact. coli* which gave a yellow colour on ordinary agar has been described by Oesterle (5). This strain appeared to be stable and did not dissociate into a form giving non-pigmented typhus-like colonies. Lipska (6) has shown that the development of alkalinity and the power of decolorizing Endo's medium is characteristic of several species of bacteria of the *coli-aerogénes* group, and hence suggests that the name *Bact. coli alkaligenes* should be suppressed. After extensive comparative study of the cultural and biochemical characters of *Bact. acidi lactici Hueppe*, Prange (7) concluded that it should be regarded as a "sport" of the *coli* group. Its iodine-fixing capacity led Barthel (8) to consider it an entirely independent species between *Bact. coli* and *Bact. aerogenes*.

The question of the survival and growth of bacteria in water supplies is of great importance, and two interesting papers on this subject have been published. Platt (9) demonstrated that when organisms of the *coli-aerogenes* group were stored in river water they declined in number—rapidly at 37° C., more slowly near 0° C.—but they survived longer in raw than in sterile river water. In water kept at room temperature and gently agitated they increased in number. On the whole the *Bact. aerogenes* type was more resistant and more likely to survive than the *Bact. coli* type except at low temperatures. Bigger (10) found coliform organisms capable of increasing in numbers when incubated at 37° C. in autoclaved water from various sources. Raw water was less good, but heating at 60° C. for 1 hr. was almost as effective as autoclaving. Similarly, filtration through a Chamberland or Pasteur filter, subjection to partial vacuum, and treatment by adsorbents such as kaolin, rendered it capable of supporting growth. Calculated in terms of nitrogen, the dry matter in water is more nutritive than peptone, but the nutritive power of the water may be destroyed by treatment with CO_2 which appears to be the inhibitory substance in raw water. This paper suggests that under certain conditions coliform organisms may increase in numbers in water—a very serious matter. Heathman *et al* (11) have shown the possibility of *Bact. typhosum* persisting even longer than *Bact. coli* in water treated with chloramine. Hence they suggest that the use of the presence or absence of coliforms as an index of the safety of water supplies needs reconsideration.

According to Malcolm (12), ordinary methods of testing demonstrate that bovine faeces contain preponderant numbers of organisms of *Bact. coli* type in summer and winter, the *Bact. aerogenes* type being rarely isolated. Using enrichment methods, the *aerogenes* type was found in 95% of the samples. He concludes that the *Bact. aerogenes* type is normally present in faeces but in very small numbers compared with the *Bact. coli* type. Pont (13) found that the numbers of *Bact. coli* strains in samples of milk from cream deliveries at factories in New South Wales were very much higher than the numbers of *Bact. aerogenes* and *intermediate* strains. It appeared, however, that the latter strains produced more serious and rapid deterioration in the quality of the cream. Similarly, Bartram & Black (14) found a preponderance of *Bact. coli* types over *Bact. aerogenes* in raw milk. They considered, however, that the *coli-aerogenes* group as a whole was a better index of contamination than any one species.

The search for a reliable selective medium for the detection and estimation of the *coli-aerogenes* group has still led only to inconclusive results. Ruchhoft & Norton (15), Farrell (16) and Horwood & Dunn (17) found that for analyses of water samples, standard lactose broth was the most satisfactory medium of the large numbers tested. Black & Klinger (18) also found the standard lactose medium satisfactory, but Hajna & Perry (19) considered Eijkman's test superior when examining sewage. The dangers involved in the addition to culture media of inhibitory substances, the toxicity of which is influenced by the protein content and the reaction of the medium, is pointed out by Stark & Curtis (20, 21), who found, however, that formate ricinoleate broth was a satisfactory medium inhibiting the growth of false test organisms. Bartram & Black (22) made extensive tests on fourteen media, none of which was completely selective. Their tests on a modified formate ricinoleate medium also gave promising results. Farrell (23), after tests on ten presumptive test media, came to the conclusion that at present no efficient medium exists. Leifson (24) considered that the enumeration of coliforms in milk and water is best made by direct count on solid media. For this he suggested desoxycholate agar, on which *Bact. coli* gives large red, and *Bact. aerogenes* colourless, colonies.

The differentiation of *Bact. coli* and *Bact. aerogenes* groups is still a vexed question. Halbert (25) has studied various organic dyes and found that Dahlia B, Janus Green B, Martius Yellow, Benzyl Violet, Aldehyde Green, gave rapid and luxuriant growth of *Bact. coli*, but completely inhibited *Bact. aerogenes*. The production of H_2S was found not to be of differential significance (26). Noble's ferrocyanide citrate agar is reported to provide a reliable test, but rather complicated for routine work (27). The changes in acidity produced by the growth of members of this group have been followed by France (28). Some of the *intermediates* resemble *Bact. coli* in this respect, some are like *Bact. aerogenes*, others cause very little or no change.

The exact position of the so-called *intermediates* has still to be defined. Tittsler & Sandholzer (29) stress the heterogenicity of the group, no single character of which will differentiate them all from *Bact. coli* or *Bact. aerogenes*. Although some of these *intermediates* may be proved to be mixed cultures, some of them, as has been shown by Kline (30), are stable in pure culture and are indivisible even by single-cell methods. The difficulties of differentiating satisfactorily between the various subgroups favour the opinion to which Kotek (31) appears to have been led—that all the *coliform-aerogenes* strains belong to one group only.

The existence of heat-resistant strains is of importance in assessing pasteurization efficiency. Henneberg & Wendt (32) found them more abundantly in cow's milk in the summer, though even then only infrequently. Their origin is still unknown, and heat resistance is difficult to maintain in cultures, being apparently a physiological condition. Of 505 pure cultures of recently isolated coliforms Stark & Patterson (33)

found none that resisted 145° F. for 30 min.[1] Hollaender & Claus (34), and Wells & Fair (35) have demonstrated the susceptibility of *Bact. coli* to ultra-violet radiation.

(*b*) Milk taints and other faults

The wide prevalence of the "oily", oxidized, or "emery" taint in milk is reflected in the large number of papers bearing on this subject. Since this taint is chemical in origin and is only indirectly influenced by the bacterial flora of the milk, the number of papers that need to be reviewed here is very small. Csiszár (36) and Thurston (37) agree in condemning the preventative measure suggested by Kende, and advocate eliminating the fault by the avoidance of bare copper and iron surfaces in the equipment and the feeding of cows on good pasture, rather than by the addition to the milk of reducing substances, even though they may be of bacterial origin. Roland *et al.* (38), in their study of the fault, repeated the observation often made by others that milk of high fat content and low bacterial count was more liable to this taint than milk of lower grades.

Davis (39) has revised and brought up to date the pamphlet issued by the Ministry of Agriculture and Fisheries on "Ropy Milk", which deals with the problem from many points of view.

Long & Hammer (40) made a full study of *Alkaligenes viscosus* and stress particularly its ability to hydrolyse fats. Amongst a large number of lipolytic organisms isolated from milk, cream, etc., were several non-ropy strains which otherwise agreed in their general characters with typical strains of *A. viscosus* and even in some cases developed ropiness on repeated culture in milk. These he considered therefore to be non-ropy strains of *A. viscosus*, analogous with non-ropy strains of *Str. lactis*. Laxa (41) has isolated and described an organism which he has named *Viscobacterium lactis foetidum*, and which was the cause of foul-smelling ropy milk. The nature of the slime and the foul-smelling product was not determined.

Olson & Hammer (42) have isolated and described two organisms, *Ps. graveolen* and *Ps. mucidolens*, responsible for a potato odour in milk. They were serious contaminants on farms where the utensils were not thoroughly cleaned and sterilized. Another species *Ps. fluorescens* has been cited by Strohecker (43) as the cause of brown milk, the colour being due to enzymic oxidation of tyrosine.

(*c*) Bacteriophage

Intimately associated with the study of bacteria is the study of bacteriophage. In spite of a vast number of researches since the original work of Twort & d'Herelle, some 20 years ago, the true nature of phage, its mode of action on bacteria, its biological significance are still undefined. This is apparent in the review (including an extensive bibliography) by Kreuger (44) of the progress made during this period. The biological complexity of bacteriophage has hindered the definition of its true nature, but it has not prevented the recognition and the study of the results of its action upon bacteria. Recent publications indicate that bacteriophage phenomena must be seriously considered in connexion with the dairy industry, since they appear to provide the explanation for hitherto obscure abnormalities of reaction.

Coliphage is the most common of those isolated from sewage. The importance to the milk industry of this phage has been specially studied by Lipska who isolated it also from milk. In a recent paper (45) dealing with examinations of winter and summer milk from the environs of Warsaw she states that the filtrates of summer milk were more active to coliform organisms than the filtrates of winter milk, and that coliform organisms from milk were more resistant to the phage than those

[1] Other references to heat resistant strains are made in the section on pasteurization.

from animal and human faeces. She puts forward the suggestion that the therapeutic value of fermented milks depends not only on the acids but also on the bacteriophages present, and raises the question of the relative quantity and activity of the phage in clean milk and milk with a high bacterial content.

There are relatively few data on streptococcal phage, it having been considered rare and difficult to isolate. Evans (46) questions this view since she has readily isolated it from sewage. Her researches were mainly concerned with four serological races of streptococcal phage active towards haemolytic streptococci, but one race was found to be active towards a strain of *Str. lactis*. Just prior to the period of this review workers in America (47, 48) had reported that the addition of bacteria-free filtrates from "slow" butter starters would delay the development of acidity in normal starters, transforming them into typical "slow" cultures. A study of the active principle of these filtrates suggested the presence of bacteriophage, although this term was not actually used. Mazé (49) correlated the "capricious" fermentation of pure starter cultures in pasteurized milk and cream with the parasitic action of a ferment not altogether identical with bacteriophage. Non-coagulating starters gave normal colonies at first, but these decreased in number until usually none was given after 48 hr. The active principle was not in the milk but was linked with the bacteria, which were not, however, lysed. Different species of lactic acid bacteria varied in their resistance to this active principle. A very similar explanation for the occurrence of so-called non-acid "starters" has been found by Whitehead and his co-workers.

Whitehead & Cox (50) described a phage isolated from apparently normal starters which, however, failed to produce normal acidity in vats of well-aerated cheese milk. The aeration appeared to be an important factor in the sudden and rapid development of the phage. A search for the sources of those strains of streptococci which, though closely resembling *Str. cremoris* or *Str. mastitidis*, possessed the capacity for producing these inhibitory substances indicated their widespread distribution but irregular occurrence (51). They should not be present in significant numbers in carefully taken and well-cooled milk supplies—they only accumulate when they are allowed to find a secondary habitat in inefficiently cleaned machines and utensils.

The importance of the discovery of bacteriophage active towards the lactic streptococci justifies its closer study. An attempt was made to control "non-acid" or "slow" starter by the isolation and propagation of phage-resistant strains of streptococci, but Whitehead & Hunter (52) found that after two or three generations the acquired resistance was lost, and secondary phages, not entirely identical with the primary, developed in the cultures. A permanent solution for the trouble could not be found by this procedure.

The development of secondary phage had not been observed previously, but Whitehead & Hunter suggest that the phenomenon is probably not peculiar to lactic streptococci and could be observed for other types of bacteria, given the necessary conditions. They consider that the results support the theory that phage is a product of the organism concerned.

These researches are only in their initial stages, but they indicate that the importance of the application of bacteriophage in the dairy and other industries is at least as great as its academic interest.

III. LACTIC ACID AND ALLIED FERMENTATIONS

(*a*) Bacterial metabolism

As adequate reviews of general bacterial metabolism have been published elsewhere (the general chemistry of bacteria has been reviewed by Stephenson (1) and the metabolism of bacteria by Kluyver (2) and van Niel (3)) only papers of interest in dairying are dealt with here.

General

Wooldridge *et al.* (4) have shown that the activity of bacterial enzymes varies with the age of the culture, increasing at first and later diminishing. In an investigation of chemical factors influencing growth and pigmentation of certain micro-organisms Kharasch *et al.* (5) have shown that traces of heavy metals may inhibit pigmentation. This inhibitory effect can be neutralized by the addition of liver, and so this tissue may inhibit the growth of organisms requiring iron, e.g. *Sarcina lutea*. A related phenomenon has been found by O'Meara & Macsween (6), who have shown that the presence of copper in peptone may result in inhibition if the medium has been heated and allowed to stand some time. Meat reactivates the peptone by removing the copper.

Respiration and oxidation-reduction potential

A useful account of the mechanisms of cellular respiration has been written by Bigwood (7), and a fourth edition of Hewitt's *Oxidation-Reduction Potentials in Bacteriology and Biochemistry* gives a very concise account of the present knowledge on this subject (8). A review of progress in the study of biological oxidations and reductions has been written by Lipmann (9).

The relation between respiration and the growth of aerobic bacteria has been studied by Hirsch (10), who has shown that oxygen uptake varies with the concentration of bacteria and after reaching a maximum finally sinks to a low level. The respiratory systems of bacteria have been investigated by many workers. Frei (11) has shown that bacteria can be grouped in seven classes on the basis of their respiratory systems (dehydrases, flavine-enzyme, peroxidase, catalase, indophenol oxidase and cytochrome). Much attention has been paid to the Warburg yellow pigment system apparently responsible for H_2O_2 production by some bacteria, and Pett (12) has investigated the lactoflavine content of some micro-organisms. Cysteine and KCN increase the flavine content, and cyanide-treated yeast is found to have a normal fermentation but a low respiration activity. A minimum phosphate concentration is required for the development of flavine, and strong aerobiosis depresses the fermentation and lowers the flavine content. It is suggested that high flavine is related to a strong fermentative ability rather than "anaerobicity".

Gillespie & Rettger (13) attribute the rapid reversion of potential during the logarithmic phase of growth of bacteria to rapid changes in the *p*H of the medium. It is claimed (14) that measurements of final potentials are sufficiently constant to permit the differentiation of lactobacilli and also of spore-forming anaerobes. Apparently even aerobes require a certain reducing intensity for growth, and Wood *et al.* (15) have found that oxidation-reduction indicators from methylene blue upwards, when in an oxidized form, inhibit the growth of *B. megatherium*. The reduced dyes and those below methylene blue did not produce any inhibition. The phenomenon was only observed in the lag phase and so appears to be due to a poising effect. Reducing agents were favourable to growth and the best zone was from 0 to

minus 0·05 v. Porter & Gillespie (16) claim that *Aerobacillus polymyxa* and *macerans* give different final potentials due to differences in carbohydrate metabolism.

Bacterial fermentations

A review of the mechanism of bacterial fermentations has been written by Kluyver (17), and an account of recent advances in the fermentation industries by Chrzaszcz & Janicki (18).

It has been known for a long time that any hexose in alkaline solution is converted partly into other sugars or at least into forms having similar chemical properties to such sugars. Wedum (19) has shown by fermentation tests that the other hexoses, usually assumed to be formed at alkaline reactions, are not present, as he failed to get positive fermentations on the addition of appropriate test organisms. Addition of the appropriate hexose resulted in normal fermentation. It is assumed that the sugar is converted to a non-fermentable form which is chemically similar to the particular hexose in question. Hotchkiss (20) found that, of a number of coliform cultures, seven strains of *Bact. coli* fermented maltose but not sucrose, and five strains of *Bact. coli communior* produced acid from sucrose but not maltose, and, in addition, six of these fermented maltose but not sucrose. He observed that toluene may change the end-products of fermentation and is not an effective bactericidal agent. Tittsler & Sandholzer (21) in an investigation of the fermentation of cellobiose found that acid and gas were produced by *Bact. aerogenes* and intermediates but not by the true *coli* group. Cocci usually failed to ferment it. Few species attacked α-methylglucoside (22), but all the *aerogenes* strains and about one-third of the *intermediates* fermented this sugar, whereas no true *coli* did so.

The conditions controlling the production of by-products are of considerable importance in the ripening of dairy products. Tikka (23), in an investigation of the effect of *p*H on *coli* fermentation, found that acidity increases lactic acid and hydrogen, decreases acetic and formic acids, and has no effect on the alcohol produced. According to Johnston & Wynne (24) the amylase of *Cl. acetobutylicum* has a *p*H optimum of 4·8–5·0 and is inert at *p*H 1·5 and 7·5. Acetate increases its activity by 25% and phosphate and chloride retard it. Bernhauer *et al.* (25) have reported that $CaCO_3$ increases the yield of butyric acid and butyl alcohol, and decreases that of acetone, acetic acid and ethyl alcohol.

Protein and fat metabolism

Moycho (26) has adduced evidence that proteases are never secreted by living bacteria but (when present) are always liberated by the autolysis of the dead cells. Gorbach & Ulm (27) have investigated the proteolytic enzymes of Gorini's acid proteolytes. The "*Mammococcus*" has a proteinase which is stimulated by KCN and has an optimum *p*H of 7. Degradations of casein and gelatine do not run parallel; thus the ageing of the culture increases caseolysis and decreases gelatinolysis. There is also present a true peptidase having a *p*H optimum of 4·8 whose activity decreases with age. Gorini (28) has demonstrated by his milk-on-agar method that rennin is present in streptococci and in *Bact. typhosum*.

Horowitz-Wlassowa & Livschitz (29) have found lipase to be present in many types of bacteria. Oxidation of fats is much less frequent than hydrolysis, but peroxides, hydroxides and aldehydes may be formed and oxygen loosely bound. Lipase and "lipoxydase" could not be detected in the medium, and bacterial lipase was found to be thermostable. The action of micro-organisms on fats has also been investigated by Jensen (30), who has shown that bacteria may induce oxidative rancidity (peroxidase and lipase), hydrolysis (lipase) and tallowiness (oxidizing enzymes). There may be a reversion of flavour and pigments may be formed and

dissolve in the fat. Pure fat does not support growth. Vickery (31) found that all the *asporogenous* yeasts and *Pseudomonas* tested, but only one of four *Achromobacterium* strains, could hydrolyse beef fat. All grew at $-1°$ C.

Physical factors

Clayson (32) has given an account of the effect of the colour and nature of light transmitted by wrapping materials on bacterial and mould growth. He concludes that a lethal effect is only evident when ultra-violet light of fairly high intensity is incident. Henneberg & Kniefall (33) have investigated the effect of sodium chloride on the growth and morphology of bacteria of importance in dairying. They found that with micrococci no effect was appreciable up to a concentration of 15%. *Str. thermophilus* was affected at 10%, *Str. faecalis* at 7%, *Str. cremoris* at 3%, and *Str. mastitidis* and *lactis* at 3–5% in agar.

Bacterial nutrition

A scholarly account of work on bacterial nutrition which deals mostly with *Staphylococcus* and *Clostridium* has been presented by Knight (34). He has further shown that the *sporogenes* growth factor may be synthesized by many micro-organisms (35). The substance stimulating the production of butyl alcohol by certain bacteria has been shown by Tatum *et al.* (36) to be asparagine. According to Regnier *et al.* (37) the source of protein is more important than the enzyme used for digestion purposes, e.g. pepsin or pancreatin. Davis (38) has shown that incubation of peptone media at 30° C. may fail to reveal the presence of heat-resistant spores and advocates "momentary autoclaving" in place of the customary three steamings. The specification of an ideal peptone is submitted, and methods for the preparation of casein double digest (which is proposed to replace peptone) given. Further, he has shown that changes in *p*H when media are heat sterilized are determined by the ratio of sugar to buffering substances (chiefly "peptone"). Litmus milk which is the most useful medium in dairy bacteriology will, by the addition of certain substances such as dextrose, yeast, blood and beerwort, give good growth of all types of significance in the study of milk products. In the presence of chalk, lactic acid bacteria remain viable in milk from 3 months upwards according to type. Finally, a standardized method for the determination of the acidity of milk is given and simple methods of adjusting media to certain *p*H values described.

Technique (with reference to metabolism)

Conn (39) has given a useful discussion of the sources of error in the detection of nitrate reduction, and the interference of lactic acid in the estimation of volatile acids in bacterial cultures has been pointed out by Allen & Harrison (40). Wedum (41) has shown that delay in fermentation may be due to the appearance of variants and organisms may utilize sugar without any evidence of acidity. The advantages of a metabolic method for the determination of sugar fermentations in the identification of bacterial species have been dealt with by Johnson (42) and Davis (43). The finding by Williams *et al.* (44) of pantothenic acid in many samples of maltose serves further to explain the apparent anomalies in sugar fermentations obtained by ordinary techniques. The difficulties of obtaining a carbohydrate-free nitrogen source are further emphasized by the work of Myrbäck & Örtenblad (45), who find that press yeast contains trehalose and a complex carbohydrate. Inoue (46) found that brain broth was good for the aerobic growth of anaerobes, as the black colour produced by some types was of differential value.

(*b*) Lactic acid bacteria

General biology

Stark & Sherman (47) have shown that *Str. lactis* can be regularly isolated from some, but not all, plants. They could not find it in the mouths or throats of cows, in bovine faeces, human faeces or soil, and suggest that plants are the natural habitat of this important type. Karnicki & Dorner (48) have investigated the lactic acid flora of rennet and found that *Tbm. lactis* was most frequent (in 85% of samples), *Tbm. helveticum* was present in 26% and *Tbm. bulgaricum* in 11%. Of the streptococci, *Str. thermophilus* was present in 88% and *Str. lactis* in 27%. A noteworthy difference between rennet and natural (whey) cultures was the presence of *Betabacterium* in 85% of the rennets but only in 12·5% of the whey cultures. Motility in the *enterococcus* has been confirmed by Pownall (49) and Koblmüller (50). Dissociation and filtration phenomena of *L. acidophilus* have been investigated by Kopeloff (51) who was unable to force "S" forms to the "R" form and was unable to get evidence for the existence of filterable forms. Foter & Rahn (52) have shown that a large number of streptococci will form acid in milk at 0° C., and that those not growing at 0° C. ferment more slowly than those that do. Thus whereas *Str. lactis* was weakened in 4–8 weeks at 0° C. *Str. faecalis* was not. *L. acidophilus* also dies out at this temperature. *Str. lactis* requires twice as much lactose as *Str. faecalis* per cell division, and *L. acidophilus* ten times as much. The largest cell crops were obtained at temperatures between the optimum and 10° lower. The metabolic activity of single cells of various strains of *Str. lactis* have been investigated by Supinska & Matuszewski (53). At the moment of reduction of methylene blue in milk the acidity has increased by 0·03–0·08% lactic acid, and the cells are in the initial stationary phase. The generation time varies from 74 to 166 min. and the fermentation activity from 4 to 24×10^{-10} g. lactic acid per cell per hour. There is a close relationship between the volume of cell and fermentation, and between the rate of proliferation and fermentation. Sherman & Hussong (54) have investigated the fermentative variability of substrains of *Str. cremoris* and *Str. lactis*. *Str. lactis* is more stable than *Str. cremoris* which latter gives many substrains fermenting maltose. Yawger & Sherman (55) have shown the existence of strains of *Str. lactis* which do not ferment lactose; these may however regain the ability to ferment lactose. (Compare ref. (112).) Bonciu (56) claims to have changed non-haemolytic streptococci to haemolytic by cultivation in polyvalent filtrates. Thus 2 of 3 "viridans" strains became β-haemolytic in 14 passages and 5 of 8 α-strains β-haemolytic in 5 to 14 passages. Once this characteristic was established it remained stable. Bier (57) has similarly claimed to have altered α-types to β-types by the action of phage. The mechanism postulated is that α-strains always contain a few β-cells and phage lyses the α- but not the β-types. He suggests that this is linked with metabolism and particularly respiration, peroxide varying inversely to haemolysin production. The resistance of enterococci to various germicides has been studied by Chapman (58) who found that they were more resistant than streptococci, staphylococci and coli-aerogenes bacteria to the action of sodium carbonate, sodium bicarbonate and sodium chloride. Enterococci were still viable after 1 hour in 3% sodium carbonate solution.

Respiration

It has been claimed by Talce-Niedra (59) that some strains of *Str. lactis* contain catalase. It may be mentioned, however, that in the study of many strains of lactic acid bacteria in this laboratory, catalase has never been found to be present in any organism true to type. Catalase may be present in cultures labelled as *Str. lactis*, etc.

but investigation invariably shows that the organism is not true to type and may be assumed to be contaminated or a mutant (60). Farrell (61) has shown that all streptococci contain a thermostable peroxidase. Since, however, this enzyme is inactivated at the reducing intensities obtaining in cultures, it would not appear to have any significance in the metabolism of the organisms. Bertho & von Zychlinski (62) have studied the oxidizing enzymes of lactic acid bacteria and state that lactic acid, alcohol, pyruvic acid and methylglyoxal are not oxidized by the Warburg system. They attempted to separate the enzyme of *L. Delbrücki* by plasmolysis and autolysis but found that the activity remained in the residue. Adler & v. Euler (63) state that free flavine is without catalytic activity. The respiration of *Tbm. helveticum* was increased by lactoflavine, ovoflavine and lumiflavine (from liver).

Wagner-Jauregg & Möller (64) conclude that the dehydrogenation of hydroxy-acids other than lactic acid requires, (1) the specific dehydrase, (2) co-enzyme, and (3) the yellow flavine, whereas that of lactic acid does not. Thus the lyochrome-free enzyme from *Bact. coli* and *Lactobacillus* was able, with methylene blue, to oxidize lactic acid. Flavine had no appreciable effect. Schlayer (65) also working with *Pneumococcus* finds that oxygen uptake varies with partial pressure, the optimum being 20–30%. He claims that growth depends on respiration as indicated by a steady increase in uptake. Cell counts and dry weights were, however, not determined. Hahn & Niemer (66) have isolated from minced muscle a thermostable substance which inhibits the aerobic production of lactic acid.

Fermentation

Further advances in the study of the lactic acid fermentation have been made in many laboratories. Chaix & Fromageot (67) have shown that sulphydril substances are necessary for bacterial fermentation. Sulphur, sulphate, thiocyanate, methylene blue, insulin and a number of other substances were found to be inactive, and $Na_2S_2O_6$ and colloidal sulphur slightly active. The mechanisms of degradation of glucose by lactic acid and other bacteria have been intensively studied in Werkman's laboratory. In collaboration with Nelson (68) he has shown that certain lactobacilli produce lactic and acetic acids, alcohol, glycerol and carbon dioxide. The latter is equivalent to the acetic acid and alcohol and the glycerol to twice the acetic acid; lactic acid may be broken down to acetic acid and carbon dioxide. Pyruvic acid is an intermediate and gives lactic and acetic acids and carbon dioxide. Under strongly reducing conditions fructose is changed to mannitol, aldehyde to alcohol and acetoin to 2 : 3 butylene glycol. Aldehyde and acetoin added to the fermentation result in an increase of acetic acid and carbon dioxide and a decrease in lactic acid, alcohol and glycerol (69). The conditions controlling the optical activity of lactic acid produced by bacteria have been studied by many workers. Kopeloff *et al.* (70) have found that the "R" form of *L. acidophilus* gives inactive lactic acid and the "R" form of *L. bulgaricus* gives inactive lactic acid at first. The "S" form of both gives the dextro-rotatory acid. Katagiri & Kitahara (71) grew lactic acid bacteria in media containing dextrose and racemic lactic acid and found that some types modified the lactic acid and others did not. Tatum *et al.* (72) have shown that *Cl. butylicum* in associated growth with lactic acid bacteria may racemize the lactic acid produced. This is due to enzyme systems consisting of intracellular thermostable and extracellular heat-labile fractions. Finkle (73) has observed that whereas inulin is strongly fermented by the *Pneumococcus* in the ordinary fermentation technique, it is neither fermented nor oxidized by this organism when the Warburg technique is used. Of the sugars studied fructose was most rapidly oxidized. Okulitch & Eagles (74) have grown streptococci in various sugar broths and then tested the effect of this treatment on their growth in milk. They obtained the rather striking result that whereas sucrose,

maltose, dextrin and starch were without effect, glucose, mannose, fructose and salicin inhibited subsequent growth in milk. They suggest that this was due to the production of "races". Similar findings to this have been reported by other workers, and it will be evident that, as this phenomenon appears to be a general one, indiscriminate use of sugar fermentation tests for the identification of bacteria without consideration of their previous history will result in confusion. Wright (75) has shown that *Str. thermophilus* may utilize lactose and sucrose more readily than other sugars on account of the more rapid fermentation of these disaccharides than of the constituent hexoses. The utilization varied according to the environment.

Nutrition

A highly interesting series of papers on the nutritional requirements of the lactic acid bacteria has appeared from Orla Jensen's laboratory. Thus it has been shown (76) that for the lactic acid bacteria of milk, colloidal casein is as good a source of nitrogen as peptone, but lactalbumin is of no value. *Thermobacterium* requires cysteine, tyrosine, lysine and histidine, *Streptobacterium* only cysteine and ammonia, and *Streptococcus* ammonia only. The subject of growth factors has also received attention (77). All the true lactic acid bacteria require pantothenic acid (a constituent of bios) and flavine. The latter cannot be replaced by glutathione. Commercial peptones have sufficient flavine but insufficient bios for the rod forms (78), and are rich in flavine on account of the enzymes used in their preparation. As whey was found to contain all the activators necessary for the lactic acid bacteria it was used by Orla Jensen in his work on the nitrogen requirements. Further data are given for other bacteria (79). The charcoal treatment of milk which removes the growth activators necessary for the lactic acid bacteria is without effect on the growth of *Sarcina flava*, *Mbm. lacticum* and *coli-aerogenes* bacteria. The tetracocci and propionic acid bacteria, however, are stimulated by bios and lactoflavine. The results of Orla Jensen *et al.* have, however, been criticized by Werkman and his collaborators on technical grounds (80). Sadler and his co-workers (81) have shown that in the case of a *Betacoccus*, alfalfa extract is a more powerful stimulant than yeast extract. Work on the fractionation of bios (82) has placed the fractions in the following order from the point of view of requirement by the Betacocci, II *a* (pantothenic acid), II *b* (vitamin B_1), I (inositol). All are, however, necessary for the best growth.

Physical factors

Reed (83) has investigated the maximum temperature of incubation permitting growth of β-haemolytic, pathogenic streptococci and found that growth does not usually take place above 41° C. and never above 44° C. A few non-pathogenic haemolytic types grew at 49·5° C. Longsworth & MacInnes (84, 85, 86) have worked on the physiology of *L. acidophilus* in cultures held at a constant *p*H. They found that the optimum *p*H is about 6 and that carbon dioxide is essential for growth. Oxidation-reduction measurements showed that the gold electrode gave the best results and that under anaerobic conditions a constant E_h of $-0{\cdot}190$ v. at *p*H 6 was obtained. If this is due to the lactate-pyruvate system it corresponds to a ratio of 100 to 1 (lactate to pyruvate). Controlled *p*H conditions gave four times the bacterial population and nine times as much acid production as in uncontrolled experiments.

Identification of lactic acid bacteria

According to Vas & Csiszár (87) the morphology and biochemical behaviour of streptococci are very variable and depend largely on environment. They claim that it is possible to change the *lactis-cremoris* group into the *paracitrovorus-citrovorus*

group by growing the organisms in whey, and also in milk with infrequent transfers or at 37° C. They suggest, therefore, that the names indicate conditions of culture rather than differences in identity. Similarly Kleckner(88) finds that the faecal and the lactic acid streptococci are very similar and denies the justification for distinguishing different types. Demeter & Pfundt(89) have studied the faecal streptococci over a period of 8 years and found that they remained remarkably constant in behaviour, especially in their sugar fermentations. Storck(90) in endeavouring to change *Str. lactis* to *Str. faecium* found that the former did not acquire heat resistance by acclimatization but, by many passages in mash, would change to *Str. faecium*. Bile did not accelerate the change. Davis(91) has discussed the characteristics and given a procedure for the isolation and identification of members of this group. This is based on morphology, growth in special litmus milk media, final acidities in milk and dextrose broth, growth at 15 and 45° C., fermentation of selected sugars, growth in bile salt lactose broth and heat resistance. General biological relationships are also discussed. A number of papers on the serological characteristics of streptococci, especially those of bovine origin, have appeared, and Plastridge & Hartsell(92) consider both biochemical and serological tests necessary. Takeda(93) has investigated the serological constitution of thirteen strains of milk streptococci (*lactis-faecalis* type) and obtained two types by agglutinin-absorption methods. The reactions brought about by lactic acid bacteria in blood agar ("haemolysis" phenomena) have been studied by Davis(94), who has shown that they cannot be used for the identification of the saprophytic types. Of the so-called haemolysis types, only β- is constant, the discoloration phenomena being very variable. The use of a classification which attaches prime importance to blood-agar reactions other than β-haemolysis is therefore unjustified. Laca & Porzecanski(95) have found that nineteen out of thirty-one strains of streptococci were both haemolytic and green-producing on blood agar. Madison(96) finds that human plasma clot was liquefied by 94% of human *Str. haemolyticus* from internal sources, by 17% from superficial sources and by only 7% of those originating from animals. Thirty-three *viridans* strains were all non-fibrinolytic. P. R. Edwards(97) has worked on the serological differentiation of haemolytic streptococci of human and animal origin. He was able to obtain differentiation by precipitin tests using acid extracts of the organisms. These gave the same results as the biochemical methods. Yawger & Sherman(98) have compared the characteristics of *Str. cremoris* with those of *Str. lactis* and shown that the former cannot produce ammonia from peptone, grow in 4% sodium chloride, at 40° C. or at *p*H 9·2, and frequently does not grow at 37° C. *Str. lactis* is positive to all these tests, and, in addition, is more tolerant to methylene blue and usually does not produce acetoin, whereas *Str. cremoris* usually does. A highly interesting *Streptococcus* which is culturally like *Str. lactis* and biochemically like *Str. paracitrovorus* has been described by Matuszewski and his collaborators(99). It has been isolated from sour potato mash and kefir. It is a very powerful producer of acetoin and diacetyl, a maximum yield being obtained at 0·9% lactic acid. The addition of citric acid increases the aroma production. It has been named by these workers *Str. diacetilactis* and is considered by them to have great possibilities in butter making. *Str. liquefaciens* has been investigated by Long & Hammer(100). It was found most frequently in ripe cheese, and its biochemical behaviour, especially from the point of view of acetoin production, has been investigated. Sherman & Wing(101) have described a haemolytic streptococcus found in milk products and named it at first *Str. haemothermophilus* and later *Str. durans*(102). The organism is apparently a haemolytic variant of *Str. faecalis*. There has been much confusion over the faecal group of streptococci, and Davis & Tarr(103) have pointed out that *Str. apis* is practically indistinguishable from *Str. zymogenes* or *glycerinaceus*, some strains

possessing the power to liquefy gelatine and peptonize milk, thus corresponding to *Str. liquefaciens*. Sherman & Hodge (104) have studied the characteristics of freshly isolated cultures of *L. bulgaricus*. These produced only 1% lactic acid in milk, were strongly anaerobic, and had a maximum temperature in most cases of 60° C. Of 200 strains, fifteen fermented sucrose, and these workers state that these strains are incorrectly described as *Tbm. lactis*. Jurnkoff (105) finds that the amount of volutin in *Tbm. bulgaricum* depends on the medium and is correlated with good growth. Hence no differentiation of *Tbm. Jughurt* from *Tbm. bulgaricum* is justifiable on this ground. Orla Jensen and his co-workers (106) have reported a thorough investigation of *L. bifidus* and *L. acidophilus* which they now call *Tbm. intestinale*. On this evidence, *L. bifidus* falls into the *Betabacterium* group, as it forms 30–50% volatile acid, has an optimum temperature of 32–38° C. and does not grow at 20 or 45° C. *Tbm. intestinale* differs by splitting casein in chalk milk, forming only traces of volatile acid and failing to give the typical branching in stab culture. This group has also been investigated by Eggerth (107). A study of *L. plantarum* has been made by Pederson (108) and of the lactobacilli of grass silage by Allen & Harrison (109). The latter were all of the *L. plantarum* type, and these authors confirmed previous findings that there exists only a very vague definition of types in this group. Pederson (110) has also reported on the gas-producing species of *Lactobacillus*. He finds that there is no one character which divides them into species, and consequently there is no justification for the large number of names in use for the members of this group.

Media

Wright (111) has studied the preparation of nutrient agar for the culture of pneumococci, streptococci and other organisms and concludes that an adequate peptone is essential. Thick layers and a low agar concentration favour large colonies. The size of these is chiefly dependent on the available foodstuffs and not on the accumulation of end-products of metabolism. Filtration impoverishes the value of broth media. Davis (112) has described special litmus milk media for the diagnostic culture of lactic acid bacteria. The latter may be placed in four groups according to their growth in plain litmus milk, dextrose litmus milk, yeast litmus milk and yeast dextrose litmus milk.

Mastitis

The bacteriological conception of mastitis which has evolved as a result of the work of Minett and his collaborators (113) has been challenged in a paper by Gibson & Muir (114). These workers studied fifty-one strains and found that these differed considerably amongst themselves, no two being identical. They also failed to confirm the value of the sorbitol and trehalose fermentation test for the differentiation of streptococci of human from those of bovine origin. Their work has been criticized by Minett (115). Stableforth (116) has dealt with the serological types of *Str. agalactiae*. Three main types are recognized, and of these *a* and *c* are most common, *a* being the more severe. The *b* type is rare but severe. He recommends slide agglutination and group precipitation (ring test) for purposes of routine identification. Hensen (117) finds that *Str. agalactiae* is a well defined species and recommends the non-reduction of litmus milk, the hydrolysis of sodium hippurate and the non-fermentation of aesculin as critical tests. Growth does not take place at 10° C. or at 45° C. and the haemolysis is never of the broad zone type but of the viridans (α) or narrow zone (weak β). Cunningham (118) has investigated the reliability of various procedures for the detection of mastitis and recommends that Minett's method should be considered as most reliable but is very laborious and not infallible. Finding that some strains reduce methylene blue in a concentration of 1 in 20,000 he suggests that

1 in 10,000 methylene blue should be used as well. Examinations are more reliable in the summer on account of the lower udder flora. Hartmann (119) has compared a number of substances from the point of view of inhibition of non-mastitis streptococci and found that whereas 0·05 % sodium azide allows mastitis streptococci to grow, 0·03 % inhibits other types. Pullinger (120) has investigated the presence of mastitis streptococci in bulk milk and has suggested methods for routine inspection. According to Bryan (121) mastitis streptococci are viable 66 days in sand, 65 days in tap water and 10 days in soil.

Propionic acid and butyric acid bacteria

Chaix (122) has reported that cystine, cysteine and glutathione are all equally effective in promoting the fermentation of dextrose by propionic acid bacteria. The best concentration of glutathione is 1 in 25,000. Fromageot & Piret (123) have found that three of six strains of propionic acid bacteria were able to grow with ammonium acetate as a source of nitrogen in the presence of dextrose. They suggest that the problems of nitrogen nutrition are dependent on growth factors rather than on complex nitrogen sources. Tatum *et al.* (124) in an investigation of the substances responsible for growth stimulation in the Neuberg precipitate fraction of potato have shown that the effect is due to ammonia and asparagine. They also attribute the stimulating action of protein hydrolysates to vitamin B_1, since this substance could replace the unknown growth stimulant (125). Langlykke & Fred (126) have investigated the mechanism of the formation of isopropyl alcohol by *Cl. butylicum*, the postulated mechanism being the reduction of acetone. Added pyruvic acid did not give, however, lactic acid, but the normal end-products. Acetoin was reduced to 2 : 3 butylene glycol and aldehyde to alcohol. The addition of hydrogen acceptors increases the amounts of isopropyl alcohol and acetone, and decreases the butyric acid and butyl alcohol.

(*c*) Fermented milks

Rosell (127) working with fermented milks finds that good yoghurt contains *Str. lactis*, *Str. thermophilus*, *L. bulgaricus* and *L. Jughurt*; cocci and rods should be about equal in numbers. In an investigation of the peptonizing bacteria of kefir (Caucasian fermented milk) Samtsevich (128) has found that the characteristic flavour is due to *Flavobacterium flavum*, *Pseudomonas viscosa* and *Proteus*. Spore-forming organisms are constantly present but few in number, and the peptonizing action stimulates the growth of *Str. lactis*.

(*d*) Starters

General

In an investigation of the factors affecting the activity of Swiss cheese starter cultures, Elliker & Frazier (129) found that fresh milk was superior to stale for the propagation of starters. Whitehead & Cox (130) found that common starters contained at least four varieties of streptococci and that the proportion of these varied even under constant conditions. If acid production was good at 20–30° C. it might be slow under practical conditions, and in the methylene blue test at 37° C. such cultures sometimes gave only feeble growth and marked involution forms; thus they were decidedly weak when grown at 37° C., and their activity fell off considerably during the "scalding" period in Cheddar cheese making, the slowness persisting afterwards. The authors therefore recommend the use of a culture which is good at 37° C. as well as at lower temperatures. (It should be noted that New Zealand practice requires much longer "scalding" at 104° F. than the English.) Using this method of selection good results were obtained, but even then cases of sudden slowness were experienced which were sometimes due to oxygen sensitivity.

Ritter & Christen(131) have confirmed Hammer's theory of the production of volatile acids and acetoin from citric acid. In considering the faults of starters they state that a starter may change to a bitter, acid condition as the result of some damage to the aroma-producing streptococci. Another fault is the production of malty flavour. They also find that the volatile acids may increase at the expense of the acetoin without perceptible change in the flavour of the starter.

Faults

Harriman(132) finds that large batch cultures are more likely to experience slowness than smaller cultures and that the fault is due to aeration. Mechanisms of oxygen sensitivity in starters have been discussed by Davis(133) from the point of view of the known data of the respiratory and fermentation mechanisms of the lactic acid bacteria. It must be mentioned, however, that Hansen(134) reports that Virtanen comes to somewhat different conclusions as to the causes of slowness. According to Virtanen abnormal milk is nearly always the cause of slowness. The postulated mechanism is the presence of germicidal substances or the lack of growth factors in the milk, and slight aeration of the milk previous to use may remove the defect by oxidation or volatilization. Considerable progress has been made by the New Zealand workers in the study of slowness in starters. Cox & Whitehead(135) have studied that form due to inhibitory streptococci and found that the highest incidence is in the summer. The responsible organisms exist in the udder of certain cows, but no correlation with udder disease could be traced. The usual sources of contamination failed to give many strains, but silage was found to be a source. Whitehead has further studied the appearance of phage in starter cultures(136). This appears to be dependent on aeration and the phages are strain specific (see also p. 375). The various factors which have been reported to be responsible for slowness in starters have been reviewed by Davis(137). Eagles *et al.*(138) find that the addition of yeast extract increases the proportion of slow acid-producing types which have a wider sugar-fermenting ability.

Aroma production

Hammer *et al.*(139) account for the disappearance of aroma in starter cultures by the reduction of acetoin to 2 : 3 butylene glycol by the "citrovorus" streptococci and also possibly by other organisms. High acidity favours the production of acetoin and delays its reduction. Low temperatures and high concentrations of sodium chloride also delay the reduction. Michaelian & Hammer(140) in an investigation of the oxidation of acetoin to diacetyl find that acetoin does not change to diacetyl in acid milk in the absence of streptococci. In cultures oxygen increases the yield of diacetyl. *Str. lactis* cannot oxidize acetoin, and so they assume the oxidation to be due to the "citrovorus" streptococci. Stahly *et al.*(141) have confirmed that the disappearance of acetoin and diacetyl in butter cultures is due to their reduction to the glycol. van Beynum(142) finds that, in association with the aroma-producing organisms, *Str. lactis* is as good as *Str. cremoris*. Vas & Csiszár(143) state that some pure *Str. lactis* strains give volatile acids and aroma (cf. ref. (98)). These workers give some useful data for a number of strains of starter streptococci. Brewer *et al.*(144) also find that diacetyl may be increased by bubbling oxygen through a culture. A similar effect is obtained by air under pressure, pressure alone being without effect. Hammer(145) has found that the addition of aldehydes to *Str. liquefaciens* in milk results in the formation of more acetoin and not the expected homologue. Formaldehyde and furfuraldehyde were without effect. He has also described a quick test for the detection of the aroma substances in butter cultures(146).

(*e*) Cheese

General manufacturing problems

Technical problems in the cheese industry have been reviewed by Davis (147) who discusses the advantages and disadvantages of large scale manufacture and advocates the adoption of *p*H determinations for the control of the cheese-making process in preference to titratable acidity measurements. Barnicoat (148) has published a useful paper dealing with the general properties of annatto and especially discoloration faults in which annatto is concerned.

Milk treatment

The effect of cooling milk on the quality of cheese has been investigated by Virtanen (149) who found that cooling for 15 hr. at from 0 to 7° C. alters the protein so that the clot is less homogeneous and less porous. He found no important changes in flora, and concluded that cooling is without objection provided it does not last for more than 3 hr. Powell (150) has reported that pasteurizing milk at 65° C. for 30 min. not only does not retard but slightly increases the clotting ability of milk. If the milk is allowed to stand, a slight retarding effect is observed. Flashing at 75° C. slightly retarded and holding for 30 min. at 75° C. definitely retarded the coagulation. Pien (151) holds that the manufacture of certain cheese is practically impossible if pasteurized milk is used. An interesting series of articles by various cheese-making experts in America on the practical aspects of the pasteurization of milk for cheese-making have been summarized (152), and the results of a questionnaire sent round to factories dealing with this problem provide some useful data (153). Funder (154) has carried out cheese-making trials with milk drawn during the last stages of lactation and finds that such milk is unfavourable. He gives as a useful criterion a yield of less than $\frac{3}{4}$ gallon per day as a practical indication that the milk has reached that stage when it is best left out of the cheese vat.

Foodstuffs and soil

An important practical problem is that of the effect of various foodstuffs on the suitability of milk for cheese making. Korolew *et al.* (155) were unable to find that the feeding of clover silage to dairy cows had any effect on the quality of the cheese. Truninger (156) concludes that the bacterial flora of soil and foodstuffs and also the quality of the latter may affect the milk from the point of view of cheese making. Heltstrand (157) found that it was impossible to produce good quality cheese if the cows were fed on large quantities of succulent feeds (e.g. beet pulp). Good quality hay gave milk producing a good cheese, and would restore the coagulability of the milk of a cow yielding non-coagulating milk. Mergner (158) found that feeding silage led to no harmful effects (but cf. ref. (163)). Zeiler *et al.* (159) investigated the influences of fertilizers on milk for cheese making and came to the conclusion that the differences in experimental results were too great to permit any conclusion to be drawn. Gorini (160) states that cheese made from "disgenetic" milk may succumb to abnormal fermentations even if no obvious faults are detectable. A safe opinion as to cheese quality can only be given after complete ripening, a matter which is unfortunately not realized by all in the cheese industry. The damaging effect of certain feeds can be neutralized by "good working" feeds. Hussmann (161) has investigated the effect of the acidity of the soil on the suitability of milk for cheese making and concludes that when cows are fed on a mineral-rich grassland they give a mineral-rich milk which is good for cheese making. Acidification of the soil has a deleterious effect on the milk from a cheese-making point of view, and hence liming is good for acid soils.

The effect of bacterial fermentations in animal foodstuffs

Van Beynum & Pette (162, 163) have studied the "blowing" of cheese due to the presence of butyric acid bacteria in silage. Laboratory experiments showed that the butyric acid bacteria did not grow at *p*H values below 4·2, although the lactic acid fermentation could take place down to *p*H 3·5. The butyric acid bacteria may grow to a certain extent in silage even when acid is added to bring the *p*H down to 3·5 on account of the uneven mixing of the acid with the silage material. They have confirmed the existence of two types of butyric acid bacteria of which only one—the lactate-fermenting type—is of importance in cheese making. Lactic acid bacteria are thus necessary for the growth of this type in the silage. They recommend the arrangement of the fodder in thin layers and the adjustment of the *p*H to below 3·5. Examination of a silage from this point of view should include the determination of lactic acid, butyric acid, the *p*H, lactic acid bacteria and butyric acid bacteria. Hostettler (164) finds that the early "blowing" of cheese is due to the *coli-aerogenes* bacteria, and the late "blowing" to butyric acid bacteria. The latter fault, if attributable to the use of silage, may persist after the cessation of feeding the silage.

The lactic acid flora

Frazier and his colleagues (165) have continued their investigations of the bacteriology of Swiss cheese and find that *Str. thermophilus* predominates at first and is later replaced by *L. helveticus* and *L. bulgaricus*. The best indication of the efficiency of the starter is the relative change in *p*H during making. They also report that the gas production by *coli-aerogenes* bacteria can be suppressed by active starters. They were unable to find that propionic acid bacteria grew in the press. Good results were obtained by the use of a starter of *L. helveticus* at 1% acidity, and these workers give the acidity data for the best quality cheese (166). An investigation of the effect of temperature (167) showed that the cheese cooled fastest just below the rind with a corresponding effect on bacterial growth and the production of acidity. Large differences in *p*H resulted in insufficient drainage and a high moisture content. In experiments on the use of *Str. thermophilus* starters (168) they found 50° C. for from 30 to 60 min. to be suitable temperature-time conditions for a preliminary ripening of part of the milk, and that such a starter was good for "dead" milk. Stolting (169) has studied the streptococci of Tilsit cheese and found a very mixed flora to be present (*Str. lactis, bovis, inulinaceus* and *glycerinaceus*). He also reports the presence of motile streptococci. Davis (170) has investigated the lactic acid flora of Cheddar cheese from the time of making up to 18 months, and found that in cheese made from clean milk the flora consists almost entirely of four types, *Str. lactis, Str. cremoris, L. casei* and *L. plantarum* and their intermediates. The cocci are replaced by rods after about a month, and after 12 months only attenuated forms of *L. casei* could be found. The factors controlling this sequence are discussed. He also found that there was a tendency for *Str. cremoris* to be more prevalent in the depth of the cheese than near the surface, and that in the first few days of ripening very vigorous strains having giant cells and producing visible proteolysis and gas in milk could be isolated. It may be of interest to observe that the most common source of *Str. liquefaciens* is ripe cheese.

Micro-organisms other than lactic acid bacteria

Bondioli (171) has described a direct method for the study of the microbial flora of cheese. Boysen (172) has studied the micro-flora of Wilster Marsch (Cheshire type) cheese and found that this consists chiefly of *Str. lactis*, the lactobacilli increasing gradually after 14 days. Micrococci were found only in the first few days. Coliforms were always present and played a definite rôle in taste but never caused "blowing".

Gas formation by coliforms in the presence of lactic acid bacteria is dependent on temperature. *Aerogenes* bacteria could cause "blowing", but these soon die during ripening. A so-called "laffiger" ("putrid, sweetish-stinking, with a bitter after-taste") flavour was due to heavy infection with *Bact. coli commune*. The bacterial and yeast flora of Camembert cheese have been investigated by Hofmann (173), who found that lactic acid bacteria were predominant together with *Oospora* and *Penicillium*. Only *Oospora* was able to produce the cheese flavour, and coliforms were always present. Kelly (174) concludes that the typical ripening of Limburg cheese is due to the surface micro-flora which consists chiefly of yeasts and *B. linens*. The biochemistry of this important bacterium has been studied by Grimmer & Schmid (175). It forms a yellow to brown pigment in Tilsit cheese and also the slimy coat in Limburg, and these authors have made a study of the protein degradation brought about by this species.

The ripening of cheese

Recent work on proteolytic enzymes has been reviewed by Sumner (176), and Parisi & De Vito (177) have studied the protein degradation in the ripening of cheese (particularly diketopiperazines and proline-peptides). Davis (178) has discussed the interrelationships of the biochemical factors concerned in cheese ripening and shown that the pink colour developed when quinhydrone is mixed with cheese may be used as a simple test for the degree of ripeness. Davies *et al.* (179) have investigated the effect of a number of chemical substances on the ripening process and conclude that Cheddar cheese is a remarkably stable system and that one of the chief factors controlling the rate of ripening is the amount of free moisture. The addition of calcium salts had a marked effect on the "body" (viscosity and modulus) of the cheese, which effect, however, disappeared after a few months' ripening. Sherwood (180) has continued his studies of the rôle of rennet in the ripening of Cheddar cheese and shown that in the presence of chloroform the same extent of protein degradation was obtained, but volatile acids and cheese flavour did not appear. In a subsequent paper (181) he reports that pepsin gave less protein breakdown than rennet. Treatment of the enzyme solutions at 38° C. and *p*H 7·25 for 10 min. and at 70° C. at *p*H 5 for 2 min. had but little effect on the proteolysis. Hansen (182) has found that *L. casei* and *Str. liquefaciens* hastened the ripening and flavour production of Cheddar cheese, and Lane & Hammer (183) found that most strains of *L. casei* improved the protein degradation, flavour and uniformity of cheese. The same authors in an investigation of the effect of pasteurizing the milk on the nitrogenous decomposition in Cheddar cheese (184) found little difference in the early stages but a faster rate of proteolysis in cheese made from raw milk at later stages of ripening. The raw milk cheese had a better flavour, those from the pasteurized milk lacking flavour and having a tough, rubbery body. Sherwood (185) has studied the action of rennet, pepsin, and trypsin on raw and pasteurized milk, especially that flash-heated at 150–160° F. He ascribes the differences obtained to changes in the non-protein part of the milk.

Moulds in cheese ripening

Ramsbottom (186) in his presidential address to the Botany Section of the British Association, 1936, has reviewed many industrial processes for which the growth of fungi is essential. Amongst the references to dairy products is one in which he states that in America one firm alone maintains 9 acres devoted to the growth of *Aspergillus niger* for the production of citric acid required by the cheese industry. An authoritative book on Gorgonzola and other blue-veined cheese has been written by Savini (187). Some of the factors influencing the growth of *Penicillium roqueforti* in cheese have been studied by N. S. Golding (188), who concludes that the removal of

CO_2 and the circulation of air accelerate growth. Demeter & Pfundt(189) have similarly studied the effect of different sources of organic nitrogen on the growth of *Penicillium*. Lane(190) has investigated the effect of eight strains of *Penicillium* on the volatile acidity and flavour of Iowa blue cheese. He found that the total volatile acidity increased during ripening and that there was present a greater proportion of the higher volatile acids in the older cheese. The different strains of mould did not differ in the type of volatile acids produced but gave different amounts and also differences in flavour. The peppery flavour could be correlated with the amount of volatile acid present. Corminboeuf(191) has similarly studied several varieties containing *Penicillium glaucum* and concludes that caproic, caprylic and capric acids are responsible for the characteristic flavour of these cheese, there being very little fundamental difference between Roquefort, Stilton, Gorgonzola and Grove City cheese. Funder(192) has prepared Roquefort cheese from goats' milk, and claims to have obtained the desirable white colour, a pliable consistency and the typical flavour of this variety.

Lane & Hammer(193) have studied the use of homogenized milk for the manufacture of blue-veined cheese of the Roquefort type. They find that such cheese ripen rapidly, have a desirable white colour and are sufficiently soft to be spread with a knife. At first a butyric odour was detected, but this was replaced by the characteristic peppery odour. The keeping quality of the cheese has not yet been studied.

Faults

Hüttig(194) has pointed out that milk must have a suitable "disposition" to allow "blowing" by aerogenes bacteria in the resultant cheese. As a convenient test he recommends the preparation of a milk agar shake culture obtained by mixing equal volumes of agar and milk and incubating for 24 hr. at from 25 to 30° C. He claims that the factor controlling this fault is not acidity, buffering value or the ratio of acid formers to alkali formers, but the presence of large numbers of growing lactic acid bacteria. The problem of undesirable fermentations in cheese has also been discussed by Platon(195), who has shown that the same bacteria will produce different effects in different milks. For the suppression of *coli-aerogenes* bacteria he recommends potassium chlorate, as it is free from the disadvantages of potassium nitrate and has no effect on the lactic acid bacteria. He recommends 2–3 g. as sufficient for 22 gall. of milk. van Beynum(196) has described a bacterial colour effect in Gouda cheese caused by an organism closely resembling, if not identical with, that producing "red spot" in Cheddar cheese. Keilling(197) has shown that red spots in Gruyère cheese may be due to the penetration of nitrate which is reduced to nitrite to produce pink coloration. Knudsen *et al.*(198) have studied the factors controlling the discoloration of cheese by saltpetre, and shown that nitrite turns pure casein brown but produces no colour change with the simpler amino acids. With tyrosine, however, a colour is produced (compare Millon's reaction). These authors conclude that the necessary conditions for this fault are contact with nitrate, the presence of bacteria which can reduce nitrate to nitrite, and a *p*H below 6. Phillips(199), studying bitter flavour in Cheddar cheese made from pasteurized milk, concludes that bitterness in the early stages of ripening can be correlated with an acidity below *p*H 5. Price(200) comes to a similar conclusion. Corbett *et al.*(201) have shown that a lactose-fermenting saccharomyces may be the cause of a gassy defect in cream cheese. Reid & Brock(202) have found that the rate of deterioration of Cottage cheese is proportional to the time and intensity of the light falling upon it. The practice of punching holes in Romano cheese to permit the readier infiltration of salt has been shown by Fabian & Severens(203) to be the cause of mouldiness, and hence they deprecate the practice. Koestler & Wegmüller(204) have shown that the characteristic smell of goats' milk

is due to caproic and caprylic acids. They were unable to separate these and assumed that in goats' milk they were associated in a complex with the fat. They have used this characteristic as the basis of a method for detecting small amounts of goats' milk in cows' milk.

Tests and analyses

Spicer & Burgwald (205) have studied the *p*H of young cheese with the view to the prediction of acid development in storage and have found that taste and feel will only predict 65% of those cheese developing this fault. The acidity developed in storage is related to the acidity at milling. These authors made *p*H determinations at 3–10 days after making and found that if the *p*H were 5·07 or greater, no acid developed in the storage. If, however, the *p*H were less than 5·07 the cheese became acid. Hiscox (206) has described the changes in the free fatty acids of Stilton cheese during ripening, and shown that in the early stages large amounts of the lower acids (chiefly acetic) are formed. With the growth of the mould, however, this volatile acidity largely disappears, and is slowly replaced by the higher acids to which may be attributed the characteristic Stilton flavour. The problem of identification of the volatile acids when present in a complex mixture, such as is present in cheese, has been studied by Davis (207), who concludes that the only specific method for individual volatile acids is that based upon the differential solubilities of the salts of heavy metals in various organic solvents. The salts of copper and iron are most suitable on account of their colour; the ease with which they hydrolyse, however, renders the method unreliable, even for qualitative work.

Mastitis in relation to cheese making

The subject of mastitis in relation to cheese making has become one of great interest, largely on account of the increase in the prevalence of mastitis in herds generally, and also on account of the large amount of inferior quality cheese which is produced. The known facts have been critically reviewed by Davis & Mattick (208), who point out that the term "mastitis milk" is used in the industry to cover many types of abnormal milk, and it cannot be said as a generalization that mastitis milk is unsuitable for cheese making without a careful definition of what is meant by the term *mastitis* in this connexion. The incidence of mastitis in its common chronic form may vary in a herd from 25 to 75%, and it will be obvious that if such milk were unsuitable for cheese making very little good cheese would be produced. Attention is drawn to the fact that milk may be by all the common chemical and bacteriological tests normal, yet fail to clot with rennet and, when mixed with other milk, interfere with the coagulation process and the growth of starter organisms. The subject has also been studied by Leitch (209), who attaches great significance to the prevalence of mastitis from the cheese maker's point of view. He states that such milk will give cheese possessing a weak body and poor flavour and that, even after the recovery of the animal, the milk will still fail to give a good cheese. Dahlberg *et al.* (210) have studied the composition of milk as affected by latent mastitis. They compared milk (1) free from infection, (2) slightly infected, and (3) with a pronounced latent infection, all three types being normal in appearance. Only slight differences in chemical composition were found (slight decrease in lactose, solids-not-fat, curd tension and specific gravity, and a slight increase in albumen and chlorine). The variations found were not greater than those that could be obtained amongst different herds.

Many papers have been published upon the curd tension of milk, especially in relation to mastitis. Sanders *et al.* (211) have investigated the curd tension of milk in relation to cheese making and shown that in 300 samples of milk the *p*H is definitely

related to the coagulation time and to the curd tension. Pasteurization softens the curd and slightly increases the coagulation time. Mastitis increases the *p*H and so also increases the coagulation time and decreases the curd tension. McDowall *et al.* (212) have shown that in addition to mastitis milk two other types of slow rennetting and soft-curd milks are found. One type is that produced by individual cows in which the characteristic persists but responds to the addition of acid. The other type is that which becomes rennet resistant and does not respond to acids. Investigation of milk from different breeds placed Ayrshire, Friesian and Jersey in order of decreasing rennetting times. Sommer & Madsen (213) find that very mild mastitis may cause a marked decrease in curd strength and an increase in rennetting time. Anderson *et al.* (214) similarly find that groups A and B streptococci and staphylococci all reduce the tension about equally and coliforms to a still greater extent. A large number of leucocytes in the milk is indicative of a soft curd. In non-mastitis milks a coefficient of correlation of +0·97 existed between the casein concentration and the curd tension, and a coefficient of +0·912 between the curd tension, calcium and phosphorus.

Processed cheese

Deger (215) states that the flora of processed cheese consists chiefly of lactic acid bacteria, micrococci, aerobic spore-formers, yeasts and moulds. The first two groups usually increase in storage. Faults in processed cheese have been studied by Caserio (216) who found that a brown coloration, bitter taste, and gas holes were due to the growth of *B. mesentericus vulgatus*. The fault was associated with a decreased acidity and fat content and a marked increase in soluble nitrogen and ammonia.

(*f*) Butter

Bacteriology in general

Demeter (217) has published a series of articles dealing with the bacteriological control of butter from the manufacturing point of view. He gives limits for *coli-aerogenes* bacteria, moulds, yeasts, caseolytic organisms and for total count in good quality butter.

Fay (218) has described a method for the microscopic examination of butter which overcomes some of the difficulties of this problem. Moir & Russell (219) have discussed the bacteriological examination of export butter and state that satisfactory products have counts as follows: Agar count, less than 50,000; lipolytic organisms, less than 1000; yeasts, less than 1000, and *coli* absent. In Queensland the following bacteriological standard is aimed at in butter fresh from the churn: total count <25,000 per g., *Bact. coli* absent in 1 g., yeasts <50 per g., moulds absent in 1 g. The cream is flash pasteurized at 180° F. or under a vacuum of 13 in. at a temperature of 190–205° F. (220) Stark & Scheib (221) have investigated the correlation between the spoilage of butter and the presence of fat-splitting and casein-digesting bacteria. They found that only Gram-negative rods were invariably present. Unsalted butter made from sweet cream, pasteurized at 74° C. for 30 min., was free from most types of spoilage. They found a good correlation between the keeping quality and the numbers of lipolytic and caseolytic organisms in the butter. The same authors have reported on the types of organism responsible for these methods of spoilage. Of 486 strains which split casein, milkfat or tributyrin and which were isolated from butter, 96 % attacked tributyrin, 77 % fat and 63 % casein. Of these strains 188 were Gram-negative rods, 151 micrococci, 74 aerobic spore-formers, 40 Gram-negative cocci, 23 sarcina and 10 Gram-positive non-spore-forming rods. The micro-biology of butter has also been studied by Wood & Thornton (222), who conclude that the yeast and mould count is inadequate as a measure of creamery sanitation and should

be supplemented by a bacterial count. In a second paper (223) they report the results of studies on the growth of moulds in and upon butter. Surface moulding is frequent, whereas interior mould growth is infrequent. Mould growth does not begin in the moisture of salted butter. The question of humidity appears to be significant, since no butter became mouldy at low humidities but all did so at high humidities. Condensation moisture thus appears to be the major factor concerned in this phenomenon. No relation could be found between the mould count and the subsequent development of mould. Parchment soaked in saturated brine appeared to be the best method of combating the fault. Riddett & Neill (224) have studied the problem of mould growth in butter boxes and Neill (225) recommends soaking the wood in a solution of sodium salicylanilide and drying. Herreid *et al.* (226) have studied the microbiology of cheese-like flavours in unsalted butter and found that raw cream usually possessed a flora which could produce a cheesy flavour in unsalted butter. The bacteria could not be detected in aseptically drawn milk, and "galactase" was not responsible for the aroma. Pure cultures could produce the flavour, although not consistently, and naturally mixed cultures were most consistent in this respect. These workers suggest that the flavours are the result of associated activity of several species, of which Gram-negative rods are the most important. The best results were obtained at 10° C. or less, and the plasma of the butter contained the necessary substances for the production of the cheesy flavours.

Flavour

Virtanen & Tarnanen (227) state that diacetyl is only formed in the presence of oxygen. Slatter (228) has studied the changes in the acetylmethylcarbinol-plus-diacetyl content of butter and found the highest content at high temperatures and high acidities (low *p*H values). These substances sometimes decreased at an early stage in the life of the butter. The *p*H of unsalted butters decreased at the higher temperatures, whereas that of salted butters remained constant. The aroma substances always decreased, but salt appeared to retard the decrease. Michaelian & Hammer (229) found that more aroma substance was present in the serum than in the butter, sweet cream giving a low yield and sour cream high values. Killed cultures of "citrovorus" streptococci did not produce acetylmethylcarbinol. Only 11–16% of added citric acid was recovered as acetylmethylcarbinol, and volatile acids were not increased by the addition of this substance. Barnicoat (230) has studied the diacetyl content of cold stored butter and finds that the starter is solely responsible. Little change takes place in cold store (14–17° F.).

Faults

Randell (231) has shown that musty flavour in butter may be due to a species of *Achromobacterium*. "Rabbito" in Australian butter is due to the growth of *Achromobacterium* (occasionally *Pseudomonas*) and occurs in both salted and unsalted butter (232). Bouska (233) in discussing the surface and tallowy flavours in butter states that the higher the flavour the more likely it is to decline, and that oxidative tallowiness is the most serious fault. Hiscox (234) has described a chromogenic fault in butter which is the result of the growth of a *Pseudomonas* which can only produce pigment at temperatures below 15° C. and in the presence of sodium chloride. Platon & Olsson (235) find that fishiness may occur if the cream is not repasteurized in the dairy in which the butter is made. Repasteurization prevents this fault and improves the butter score. Vernon (236) has reported some mycological investigations of butter and described the following types of surface moulding: (1) bluish black spots (*Cladosporium herbarum*); (2) spreading muddy brown areas (*Phoma*); (3) green

surface growth (*Penicillium* or *Aspergillus*); (4) small black spots (*Stemphylium*); (5) large orange-yellow patches; and (6) large reddish pink areas (*Fusarium*). The growth of these moulds is largely dependent on temperature, humidity and foodstuffs in the wrapping material. Internal discoloration is usually due to *Cladosporium herbarum*. Slight growth may take place at −4 to −6° C. but not at −7 to −9° C. Salt retards growth. Mycological problems have also been discussed by Vernon (237) in a further series of papers.

Analysis and tests

The application of the catalase test to butter has been studied by Cruess-Callaghan (238), who found a correlation coefficient of −0·4 between the catalase figure and keeping quality. Storen & Nestaas (239), however, conclude that this test is not reliable. Sorkin (240) obtained concordant results between the methylene-blue reductase and the agar plate-count method for the determination of the bacteriological quality of buttermilk.

Keeping quality

Overman (241) in an interesting paper on the keeping quality of butter asserts that the judge's score is no indication of keeping quality. He gives interesting figures for the effect of ripening, neutralizing, etc. Guthrie *et al.* (242) have studied the effect of certain factors upon keeping quality, and conclude that pasteurization of cream at 74° C. for 30 min. destroys most if not all of the harmful enzymes. A temperature of 63° C. is not sufficient. The enzymes were not retarded by salt but were inhibited at *p*H 4·7. All non-spore-forming organisms were killed at 74° C. Gilmour & Arup (243) find that the keeping quality of butter in cold store is poor if the *p*H is less than 6·7. A butter with a high flavour and a high *p*H keeps well, but one with a low flavour and a high *p*H keeps better. Kellermann (244) has compared judging and bacterial analysis in 265 tests and obtained agreement in 72% of these. Ause & Macy (245) could find no correlation between the numbers of *Oospora lactis* and the keeping quality after storage. This organism could, however, destroy the flavour of fine starter butter.

IV. PASTEURIZATION AND OTHER PROCESSES

The use of pasteurization as a ready means of ensuring a "safe" milk supply and incidentally of improving the keeping quality of the milk is continually extending. Hiscock (1), at a meeting of the International Association of Dairy Inspectors, again laid stress on the need for educating the general public in the value of milk as a food, and of pasteurization as a means of safeguarding its quality. At the same time it was at least as essential to educate the farmer in the necessity for the production of clean and good milk, an inevitable preliminary to successful pasteurization. Milk sold at the farms will not be subjected to commercial pasteurization and may therefore be a potential source of danger to rural populations. Bryan *et al.* (2) advocate the home pasteurization of such supplies and recommend the use of a double boiler as a safe and practical method.

The high temperature short-time process still commands attention from the scientific as well as the practical point of view. It seems that fewer new patents have been taken out during the period under review, and these are mainly concerned with improvements in existing designs rather than with the development of new, indicating that the method is rapidly emerging from the experimental stage and becoming a more practical proposition. There seems to be a decided trend towards the comparison of the total effect of the high-temperature short-time process with that of

holder pasteurization. It is difficult to compare the two methods under commercial conditions, for whilst it is possible in a continuous-flow system to obtain a raw-milk sample which is reasonably comparable with the pasteurized, it is very difficult to do so when the flow is discontinuous, as in the holder process. This was realized by Caserio (3) when attempting to compare the efficiency of a Stassano plant in one dairy with that of an A.P.V. Holder plant in another. With this reservation, both processes seemed to be equally good as regards the effect on the creaming capacity of the milk and its value for cheese making. The treated milk appeared to have a slight advantage in flavour and keeping quality and probably also in total bacterial content, but both treatments freed the milk from living coliforms. The author concluded that the "stassanizing" process was well worth serious consideration, an opinion shared by others for the high-temperature short-time process as a whole.

The efficiency of this process in destroying pathogenic organisms is reiterated in a report issued by the National Institute for Research in Dairying (4), where it is stated that milk heavily infected with tubercle bacilli and haemolytic streptococci was rendered non-infective for guinea-pigs by heating at 160° F. for 15 sec. Mattick & Hiscox have also tested numerous samples from a commercial stassanizing plant but did not find tubercle bacilli in any, although on five occasions these organisms were present in the corresponding raw-milk samples (5). Meyn (6), examining the milk from two plate pasteurizers in Leipzig, in which the milk was heated at 160–165° F. (71–74° C.) for 31 sec., found neither tubercle bacilli nor *Br. abortus* in the pasteurized milk, although 50% of the raw-milk samples were infected with tubercle bacilli and 29·16% with *Br. abortus*. Bertucci (7) was also able to demonstrate the destruction of pathogenic organisms in a Stassano plant. Lembke & Claussen (8) found that the rate of flow of the milk through the heater, i.e. the rate of rise of temperature, had very little effect on the efficiency of a short-time heater. On the other hand, the length of the holding period had a marked influence on the decrease in the number of viable micro-organisms and on the destruction of coliforms. Yale (9) has summarized the contents of various papers (most of which were noted in the last Biennial Review on Dairy Bacteriology) dealing with high-temperature short-time pasteurization, and discusses their significance especially with respect to conditions in U.S.A. Four types of plant (the Stassano plant was not included) were tested and all gave satisfactory results.

Judging from the results now available there seems every reason to believe that high-temperature short-time pasteurizing plants run with reasonable care will, under commercial conditions, provide a milk supply free from pathogenic organisms and of good keeping quality. Official recognition of this method is, however, very tardy. The Illinois amended pasteurization law (10) now allows this process, provided the milk is heated at 160° F. (71·7° C.) or above for not less than 15 sec. Several States of America now officially recognize this process, and that country is therefore probably in a better position than any other to assess its true value in comparison with the well-established Holder process. Putnam (11) reviewed the situation in a paper read at the 28th Annual Convention of the International Association of Milk Dealers, and pointed out that although for various reasons the high-temperature short-time process continues to gain ground, there are some factories which have returned to the Holder process after giving the other a trial. The question of the superiority of either process is far from arriving at a satisfactory settlement. Each appears to have its own advantages and to be especially suitable for certain purposes.

Whichever process is adopted efficient supervision and control are essential. Chemical and bacteriological tests are not infallible indicators of the amount of heat treatment the milk has received. The first essentials are intelligent operation and strict supervision of the plant. Reports on observations of various plants have

revealed some illuminating facts. Taken on the whole the majority of plants are efficient and honestly operated, but even so, improvements in minor details could with advantage be effected. The need for adequate control has been stressed by several writers (12–19), and as a corollary to a survey of pasteurizing plants in four Scottish cities Scott & Wright (20) have advocated (as others have done before) the training and certification of plant supervisors and operators.

Laboratory routine control of pasteurized milk is valuable, but bacteriological tests do not confirm or disprove the destruction of pathogenic organisms, unless they are supplemented with other special tests. The phosphatase test of Kay & Graham (21) has the great advantage of being sensitive at the temperature of pasteurization (holder) and thus will reveal heating at a temperature $1\frac{1}{2}$° F. below the minimum, heating at 145° F. for 20 min. instead of 30, or an admixture of 0·25 % raw milk to properly pasteurized milk. As far as has been ascertained, the test appears to be equally sensitive for high-temperature short-time pasteurization. It has proved very valuable in detecting deficiencies in pasteurization plants, and gives the best rapid indication of the possible survival of pathogenic organisms. Microscopical examination of milk film is often recommended as a test for pasteurized milk. Helmreich (22) has observed, however, that raw milk may appear to be free from cells and streptococci, whereas the same milk pasteurized might be condemned on this test. This observation has been confirmed by Eble & Pfeiffer (23). The reason for this is not clear, and further researches are necessary before it can be stated with certainty what change it was that released these cells from the state of combination in which they were present in the raw milk.

Most pathogenic organisms are destroyed by pasteurization at 145° F. for 30 min. Slanetz (24) has, however, called attention to the prevalence of haemolytic streptococci in properly pasteurized milk, and Breed *et al.* (25) have reviewed the literature dealing with the occurrence of heat-resistant micrococci in the same medium; but the pathogenic or even the Public Health significance of neither group has been established. The origin and identity of the former are still under investigation, the latter appear to be mainly of significance in throwing light on the conditions of milk production.

The value of the presumptive coliform test in the laboratory control of pasteurization process is still a subject of controversy. The undoubted existence, though rare, of heat-resistant strains, prevents its use as an absolute test for full heat treatment (26, 27). Demeter & Eisenreich (28) point out that there is an agent in raw milk which lowers the resistance of these strains, but it is not equally active in all milks and at all times. Vernon & Walker (29) have found heat-resistant strains so infrequently in pasteurized milk that they consider their importance from the point of view of plant management to be negligible.* Several writers reassert the value of the test as a sign of recontamination after pasteurization, and therefore an indication of faulty plant management. Chilson & Yale (30), and Chilson *et al.* (31) have used the coliform test with great success in detecting contamination in the pasteurizing plant, but consider it should be used to supplement and not supplant the plate count. Graham (32) has attempted to introduce refinements into the presumptive coliform test but could find no more suitable test organisms than those of the *coli-aerogenes* group. Alexander & Milone (33) emphasize that the coliform test cannot be used to confirm correct time and temperature limits, but it does indicate the efficiency of the pasteurization process as a whole. Forgacs (34) also insists that although the test may not be absolute because of the existence of heat-resistant strains of *Bact. coli* it is a necessary aid in the bringing of the pasteurized milk supply to a high grade.

* Other references to heat-resistant strains are found in the section "*Coli-aerogenes*", p. 372.

Many objections may be raised to the heating of milk as a means of extending its keeping quality. Various methods may be employed for the destruction of micro-organisms, but those which do not rely on toxicity and can therefore be applied to the preservation of foodstuffs are relatively rare. The application of extreme cold may have an inhibitory effect on the growth of bacteria, but its lethal action varies. Thus Haines(35) found that whilst the maximum death rate of *Ps. pyocyaneus* in the frozen state occurred at −2° C., *Staph. aureus* and spores of various bacteria were able to survive rapid freezing to −70° C. The response in other vegetative cells varied correspondingly. The U.S. Bureau of Dairy Industry has patented a process(36) in which milk is concentrated to half or one-third of its original volume, when it can be frozen without injury to its physical properties. It can be held and transported in this frozen state, and when needed thawed and brought to its original volume by adding water.

The use of sonic energy has not yet been placed on a commercial basis for the treatment of milk. Beckwith & Weaver(37), using Wood & Loomis' apparatus, showed that various yeasts and bacteria (including spores) were destroyed in water or watery solutions. In milk the results were irregular, the destruction of bacteria was less effective than in tap water. It appeared that the protein interfered with the germicidal effect of supersonic treatment. A description has been given(38) of an apparatus in which electricity is used to control the acidity of milk. A direct current causes electrolysis of the acid in the milk. By regulating the current and speed of passage of the milk, it is said that the final acidity can be controlled, but figures are not given. The germicidal action of ultra-violet light and other irradiations on cultures of bacteria has been studied, but no fresh light has been thrown on the adaptation of the process to the sterilization of milk.

Ozone has an inhibitory effect on the growth of bacteria. Haines(39) has found that the growth of *Bact. coli* is retarded by the presence of 4 p.p.m. in the atmosphere and prevented by 10 p.p.m. if the ozone is admitted at the time of inoculation. Once growth is established more than 200 p.p.m. are needed to arrest it.

The Hofius process of preserving milk, in which milk placed in strong metal containers is stored under oxygen at a pressure of 8–10 atmospheres, has attracted attention. If the milk is stored at temperatures below 8° C. its period of sweetness is very much prolonged. Heine(40) has found that such milk kept sweet for 60 days—in fact its flavour was improved as a result of the removal of stall odours. Van Raalte(41) found raw milk and pasteurized cream so treated still fresh after 5 months, but pasteurized milk not after 3 months. The process may prove to be of considerable economic importance. Mattick(42) found that the bacterial count of the stored milk decreased initially, but eventually showed a gradual increase. At first there was no change in acidity, but later there was a rise. Coliform organisms appeared to be unable to survive. Müller(43) tested milk which had been artificially infected with cultures of organisms, and found that Proteus and coliforms were not killed by the process, although the former were much attenuated after 14 days. Data of the results of his studies on pathogens are not yet available. A drawback to the process is that storage at temperatures below 8° C., which is an essential feature, is scarcely practicable in hot climates. A modification suggested by Richter(44) in which the milk is preheated at 58° C. for 3 hr. before filling into the containers obviates the need for low-temperature storage and renders pasteurization superfluous. Data on the results of this modification are not yet available.

The process does not appear to be viewed with great favour in America. Early studies at Geneva are recalled(45) when CO_2 under pressure was used with very similar results, suggesting that pressure and not oxygen is the important factor. Harding(46) points out the disastrous effects that would follow the explosion of a

container with such a high internal pressure. He also considers that the milk is not "safe" from the health point of view unless it is previously pasteurized. Da Silva-Mello (47) fears that the high oxygen pressure must result in the destruction of the antiscorbutic vitamins. On the other hand, the Kiel report indicates that the process offers great possibilities in maintaining the nutritive values (44).

The process of "canning" is used to prolong the life of suitable dairy products, but even when high temperatures are used bacteria not infrequently survive and cause deterioration of the product. Nichols (48) has reviewed the various faults that may occur in sweetened condensed milk, evaporated milk and canned cream, indicating the micro-organisms that may be responsible for each. Olson & Hammer (49) have confirmed the findings of earlier workers, that two types of yeast are responsible for gas production in tins of sweetened condensed milk, and have amplified the descriptions of these organisms. Blowing in tins of sweetened milk preserves was traced by Damm & Hornbostel (50) to the action of betabacteria, in all probability introduced with the fruit juices. Normally the fruit juices were not heated, and it was suggested that pasteurization at 70–72° F. for ½ hr. would be a remedy. Cases of bitterness in canned cream were shown by McMaster (51) to be due to the presence of a spore-bearing organism closely related to *B. cohaerans*. Raising the canning temperature appeared to end the trouble. Black spots on the tins (52), and discoloration and corrosion in canned cream (53), were found to be due to chemical reactions.

The more extensive manufacture of ice cream has focused attention on the need for more stringent control of the manufacture and sale of this product. Hitherto the regulations have applied more particularly to the chemical purity and quality of the mix, but it is becoming increasingly urgent that sanitary control should be exercised too. This point of view has been expressed by Thomas (54) and Anderson (55) in England, and by Fabian (56) and Sommer (57) in America as well as by others. The desirability and also the difficulty of imposing bacteriological standards is realized. Fisher (58) stressed the fact that bacterial counts can only be estimated, and suggested that the bacterial standards should be in the form of regulations and should embody plant supervision and control, a view supported by many others. Grimes (59) and Babel (60) found tryptone glucose agar the most satisfactory medium for plate counts of ice cream. The latter studied the types and relative numbers of organisms in ice cream of various kinds. Fay (61) found that the presumptive coliform count gave the same information in general as the plate count, but the advantages were not sufficiently obvious to justify its use in routine analysis. Martin (62) advocates pasteurization of the mix to 150° F. for 30 min. as a safeguard. After-contamination should be studiously avoided.

In recent years the movement towards standardization of method and technique in the examination of dairy products which started many years ago has been accelerated. In spite of difficulties there is a marked desire to create standards for products other than milk, e.g. ice cream and butter. As in other fields of bacteriology the study of bacterial development, growth factors and variation has been intensified. There is also a welcome tendency towards simplification and contraction of classification and the adoption of a more reasonable attitude regarding the so-called "intermediate" forms. The important group of lactic acid bacteria continues to be studied extensively, and progress has been made in the definition, classification and biochemistry of the members of this group, especially in their relation to dairy products. The study of bacteriophage has now extended to this group and has assumed practical significance. The contributions from dairy bacteriologists promise to add to our understanding of the mechanism of the phenomenon. There has been less work carried out on pasteurization which is now well established and accepted, but in countries

where high-temperature short-time processes are not officially recognized when used for milk for human consumption, efforts to prove their efficacy continue. The trend of experimentation is now directed rather to other processes, amongst which the use of sonic energy and of gases under pressure is prominent.

A. T. R. MATTICK
E. R. HISCOX
J. G. DAVIS

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING,
UNIVERSITY OF READING

REFERENCES

I. MILK PRODUCTION AND CONTROL

(1) WILSON *et al.* (1935). *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 206.
(2) MATTICK *et al.* (1935). *J. Dairy Res.* **6**, 130.
(3) BOWERS & HUCKER (1935). *Tech. Bull. N.Y. agric. Exp. Sta.* No. 228.
(4) HISCOX *et al.* (1932). *J. Dairy Res.* **4**, 105.
(5) YALE & PEDERSON (1936). *Amer. J. publ. Hlth*, **26**, 344.
(6) DEMETER (1936). *Milchw. Forsch.* **18**, 131.
(7) NICHOLS & EDWARDS (1936). *J. Dairy Res.* **7**, 258.
(8) MALCOLM & LEITCH (1936). *Scot. J. Agric.* **21**, 4.
(9) THORNTON, SANDIN & MILLER (1935). *Canad. J. Res.* **13**, Sect. B, 257.

II. MICRO-ORGANISM IN MILK AND MILK PRODUCTS

(1) DOMBROWSKY (1936). *Zbl. Bakt.* I. Orig. **137**, 160.
(2) RENNEBAUM (1935). *J. Bact.* **30**, 625.
(3) TORREY & MONTU (1936). *J. Bact.* **32**, 329.
(4) BRUSCHETTINI (1936). *Ann. Igiene*, No. 1 (*Boll. Sez. ital. Soc. int. Microbiol.* **8**, 132, 1936).
(5) OESTERLE (1935). *Zbl. Bakt.* I. Orig. **134**, 115.
(6) LIPSKA (1935). *Lait*, **15**, 705.
(7) PRANGE (1935). *Zbl. Bakt.* II. **92**, 305.
(8) BARTHEL (1936). *Lantbrukshögskolans Ann.* **3**, 179.
(9) PLATT (1935). *J. Hyg., Camb.*, **35**, 437.
(10) BIGGER (1937). *J. Path. Bact.* **44**, 167.
(11) HEATHMAN, PIERCE & KABLER (1936). *Publ. Hlth Rep.* **51**, 1367.
(12) MALCOLM (1935). *J. Dairy Res.* **6**, 383.
(13) PONT (1935). *J. Dairy Res.* **6**, 148.
(14) BARTRAM & BLACK (1937). *Food. Res.* **2**, 21.
(15) RUCHHOFT & NORTON (1935). *J. Amer. Wat. Wks Ass.* **27**, 1134 (*Brit. Chem. Abstr.* B, No. 1071, 1935).
(16) FARRELL (1936). *J. Bact.* **31**, 19.
(17) HORWOOD & DUNN (1936). *J. Bact.* **31**, 20.
(18) BLACK & KLINGER (1936). *J. Bact.* **31**, 171.
(19) HAJNA & PERRY (1935). *J. Bact.* **30**, 479.
(20) STARK & CURTIS (1936). *J. Bact.* **32**, 375, 385.
(21) —— —— (1936). *Amer. J. publ. Hlth*, **26**, 354.
(22) BARTRAM & BLACK (1936). *Food Res.* **1**, 551.
(23) FARRELL (1937). *J. Dairy Sci.* **20**, 67.
(24) LEIFSON (1935). *J. Path. Bact.* **40**, 581.
(25) HALBERT (1935). *J. Bact.* **30**, 653.

(26) Vaughn & Levine (1936). *J. Bact.* **32**, 65.
(27) Hoak (1935). *J. Penn. Wat. Wks Op. Ass.* **7**, 75 (*Chem. Abstr.* **30**, 3016, 1936).
(28) France (1936). *J. Bact.* **32**, 125.
(29) Tittsler & Sandholzer (1935). *J. Bact.* **29**, 349.
(30) Kline (1935). *Amer. J. publ. Hlth*, **25**, 833.
(31) Kotek (1935). *Ann. Soc. bot. Pol.* **10**, 537 (*Zbl. Bakt.* II. **94**, 351, 1936).
(32) Henneberg & Wendt (1935). *Zbl. Bakt.* II. **93**, 39.
(33) Stark & Patterson (1936). *J. Dairy Sci.* **19**, 495.
(34) Hollaender & Claus (1936). *J. gen. Physiol.* **19**, 753.
(35) Wells & Fair (1935). *Science*, **82**, 280.
(36) Csiszár (1935). *Öst. Milchw. Ztg*, **42**, 41 (*Chem. Abstr.* **29**, 3413, 1935).
(37) Thurston (1935). *Proc. int. Ass. Milk Dlr*, **28**, Lab. sect., p. 121.
(38) Roland, Sorensen & Whitaker (1937). *J. Dairy Sci.* **20**, 213.
(39) Davis (1935). *J. Minist. Agric.* **42**, 658.
(40) Long & Hammer (1936). *Iowa St. Coll. J. Sci.* **10**, 261.
(41) Laxa (1936). *Zbl. Bakt.* II. **95**, 125.
(42) Olson & Hammer (1934). *Iowa St. Coll. J. Sci.* **9**, 125.
(43) Strohecker (1934). *Dtsch. NahrMittRdsch*, p. 130 (*Chem. Abstr.* **30**, 2650, 1936).
(44) Krueger (1936). *Physiol. Rev.* **16**, 129.
(45) Lipska (1936). *Proc. II. Int. Congr. Microbiol., London*, p. 188.
(46) Evans (1934). *Publ. Hlth Rep.* **49**, 1386.
(47) Anonymous (1933). *Rep. Ia agric. Exp. Sta.* p. 63
(48) Harriman (1934). *Iowa St. Coll. J. Sci.* **9**, 155.
(49) Mazé (1936). *C.R. Soc. Biol., Paris*, **123**, 565.
(50) Whitehead & Cox (1936). *J. Dairy Res.* **7**, 55.
(51) Cox & Whitehead (1936). *N.Z. J. Agric.* **52**, 38.
(52) Whitehead & Hunter (1937). *J. Path. Bact.* **44**, 337.

III. Lactic acid and allied fermentations

(1) Stephenson (1935). *Ann. Rev. Biochem.* **4**, 593.
(2) Kluyver (1936). *Ann. Rev. Biochem.* **5**, 539.
(3) van Niel (1937). *Ann. Rev. Biochem.* **6**, 595.
(4) Wooldridge, Knox & Glass (1936). *Biochem. J.* **30**, 926.
(5) Kharasch, Conway & Bloom (1936). *J. Bact.* **32**, 533.
(6) O'Meara & Macsween (1936). *J. Path. Bact.* **43**, 373.
(7) Bigwood (1935). *Le mécanisme de la respiration cellulaire en aerobiose et en anaerobiose.* Paris: Masson et Cie.
(8) Hewitt (1936). *Oxidation Reduction Potentials in Bacteriology and Biochemistry*, 4th ed. London County Council.
(9) Lipmann (1937). *Ann. Rev. Biochem.* **6**, 19.
(10) Hirsch (1936). *Rev. Fac. Sci. Univ. Istanbul*, **1**, 44 (*Brit. chem. Abstr.* A, 897, 1936).
(11) Frei (1935). *Zbl. Bakt.* I. Orig. **134**, 26.
(12) Pett (1935). *Biochem. J.* **29**, 937.
(13) Gillespie & Rettger (1936). *J. Bact.* **31**, 14.
(14) —— (1936). *J. Bact.* **31**, 101.
(15) Wood, Wood & Baldwin (1935). *J. Bact.* **30**, 593.
(16) Porter & Gillespie (1937). *J. Bact.* **33**, 112.
(17) Kluyver (1935). *Ergebn. Enzymforsch.* **4**, 230.
(18) Chrzaszcz & Janicki (1936). *J. Soc. chem. Ind., Lond.*, **55**, 884.
(19) Wedum (1936). *J. Bact.* **32**, 175.
(20) Hotchkiss (1935). *J. Bact.* **29**, 391.

(21) Tittsler & Sandholzer (1936). *J. Bact.* **31**, 301.
(22) —— —— (1935). *J. Bact.* **29**, 363.
(23) Tikka (1935). *Ann. Acad. Sci. fenn.* A, **41**, No. 4 (*Chem. Abstr.* **30**, 4536).
(24) Johnston & Wynne (1935). *J. Bact.* **30**, 491.
(25) Bernhauer *et al.* (1936). *Biochem. Z.* **287**, 61.
(26) Moycho (1935). *C.R. Acad. Sci., Paris*, **201**, 859 (*Chem. Abstr.* **30**, 1832, 1936).
(27) Gorbach & Ulm (1935). *Arch. Mikrobiol.* **6**, 362.
(28) Gorini (1935). *Milchw. Forsch.* **17**, 87; *Zbl. Bakt.* I. Orig. **135**, 275.
(29) Horowitz-Wlassowa & Livschitz (1935). *Zbl. Bakt.* II, **92**, 424.
(30) Jensen (1937). *J. Bact.* **33**, 98.
(31) Vickery (1936). *J. Coun. sci. industr. Res. Aust.* **9**, 196.
(32) Clayson (1936). *Congr. R. sanit. Inst.* 167.
(33) Henneberg & Kniefall (1935). *Milchw. Forsch.* **17**, 146.
(34) Knight (1936). *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 210.
(35) —— (1936). *Proc. II. Int. Congr. Microbiol., London*, p. 441.
(36) Tatum, Peterson & Fred (1935). *J. Bact.* **29**, 563.
(37) Regnier, David & Morchoisne (1935). *C.R. Soc. Biol., Paris*, **120**, 415.
(38) Davis (1936). *Ann. Conf. Soc. agric. Bact.* "Notes on the preparation of media."
(39) Conn (1936). *J. Bact.* **31**, 225.
(40) Allen & Harrison (1935). *Biochem. J.* **29**, 2471.
(41) Wedum (1936). *J. infect. Dis.* **58**, 234.
(42) Johnson (1935). *Science*, **81**, 620.
(43) Davis (1936). *Proc. II. Int. Congr. Microbiol., London*, p. 62.
(44) Williams *et al.* (1936). *Biochem. J.* **30**, 2036.
(45) Myrbäck & Örtenblad (1936). *Biochem. Z.* **288**, 329.
(46) Inoue (1935). *J. Orient. Med.* **22**, 1011 and 1029 (*Bull. Hyg.* **10**, 660).
(47) Stark & Sherman (1935). *J. Bact.* **30**, 639.
(48) Karnicki & Dorner (1934). *Ann. agric. Suisse*, **9**, 1079 (*Lait*, **16**, 633, 1936).
(49) Pownall (1935). *Brit. J. exp. Path.* **16**, 155.
(50) Koblmüller (1935). *Zbl. Bakt.*, I. Orig. **133**, 310.
(51) Kopeloff (1934). *J. infect. Dis.* **55**, 368.
(52) Foter & Rahn (1936). *J. Bact.* **32**, 485.
(53) Supinska & Matuszewski (1936). *Zbl. Bakt.* II, **94**, 499.
(54) Sherman & Hussong (1937). *J. Dairy Sci.* **20**, 101.
(55) Yawger & Sherman (1937). *J. Dairy Sci.* **20**, 83.
(56) Bonciu (1935). *C.R. Soc. Biol., Paris*, **119**, 214.
(57) Bier (1935). *C.R. Soc. Biol., Paris*, **120**, 172.
(58) Chapman (1936). *J. Bact.* **32**, 41.
(59) Talce-Niedra (1936). *Lait*, **16**, 225.
(60) Davis (1933–7). National Institute for Research in Dairying, unpublished observations.
(61) Farrell (1935). *J. Bact.* **29**, 411.
(62) Bertho & von Zychlinski (1934). *Liebigs Ann.* **512**, 81 (*Biol. Abstr.* **9**, 1339, 1935).
(63) Adler & von Euler (1934). *Hoppe-Seyl. Z.* **225**, 41.
(64) Wagner-Jauregg & Möller (1935). *Hoppe-Seyl. Z.* **236**, 216 (*Chem. Abstr.* **30**, 494).
(65) Schlayer (1936). *J. Bact.* **31**, 181.
(66) Hahn & Niemer (1936). *Z. Biol.* **97**, 195 (*Physiol. Abstr.* **21**, 515, 1936).
(67) Chaix & Fromageot (1937). *Enzymologia*, **1**, 321.
(68) Nelson & Werkman (1936). *J. Bact.* **31**, 4; (1935), **30**, 547.
(69) —— —— (1936). *J. Bact.* **31**, 603.
(70) Kopeloff *et al.* (1937). *J. Bact.* **33**, 89.
(71) Katagiri & Kitahara (1936). *J. agric. chem. Soc. Japan*, **12**, 96 (*Chem. Abstr.* **30**, 4532, 1936).

(72) Tatum, Peterson & Fred (1936). *Biochem. J.* **30**, 1892.
(73) Finkle (1936). *J. Bact.* **32**, 473.
(74) Okulitch & Eagles (1936). *Canad. J. Res.* B, **14**, 320.
(75) Wright (1936). *J. Path. Bact.* **43**, 487.
(76) Orla-Jensen, Otte & Snog-Kjaer (1936). *Zbl. Bakt.* II, **94**, 460.
(77) —— —— —— (1936). *Zbl. Bakt.* II, **94**, 434.
—— —— —— (1936). *Mem. Acad. R. Denmark*, ser. 9, VI, **5**.
(78) —— —— —— (1936). *Zbl. Bakt.* II, **94**, 452.
(79) —— —— —— (1936). *Zbl. Bakt.* II, **94**, 447.
(80) Wood, Anderson & Werkman (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 217.
(81) Sadler *et al.* (1936). *Canad. J. Res.* B, **14**, 139.
(82) Eagles, Wood & Bowen (1936). *Canad. J. Res.* B, **14**, 151.
(83) Reed (1936). *J. Bact.* **31**, 104.
(84) Longsworth & MacInnes (1936). *J. Bact.* **32**, 567.
(85) —— —— (1935). *J. Bact.* **29**, 595.
(86) —— —— (1936). *J. Bact.* **31**, 287.
(87) Vas & Csiszár (1936). *Milchw. Forsch.* **18**, 68.
(88) Kleckner (1935). *J. Lab. clin. Med.* **21**, 111 (*Zbl. Bakt.* I. Ref. **122**, 14, 1936).
(89) Demeter & Pfundt (1935). *Milchw. Forsch.* **17**, 44.
(90) Storck (1936). *Zbl. Bakt.* II, **95**, 284.
(91) Davis (1936). *Ann. Conf. Soc. agric. Bact.* "A procedure for the isolation and identification of the lactic acid bacteria."
(92) Plastridge & Hartsell (1937). *J. Bact.* **33**, 24.
(93) Takeda (1935). *Z. ImmunForsch.* **86**, 347 (*Zbl. Bakt.* I. Ref. **121**, 22, 1936).
(94) Davis (1936). *Ann. Conf. Soc. agric. Bact.* "The 'haemolysis' phenomena of lactic acid bacteria in blood agar."
(95) Laca & Porzecanski (1936). *C.R. Soc. Biol., Paris*, **121**, 558.
(96) Madison (1935). *Proc. Soc. exp. Biol., N.Y.*, **31**, 1018.
(97) Edwards (1935). *Res. Bull. Ky agric. Exp. Sta.* No. 356.
(98) Yawger & Sherman (1937). *J. Dairy Sci.* **20**, 205.
(99) Matuszewski, Pijanowski & Supinska (1936). *Rocz. Nauk rol.* **36**, 1.
(100) Long & Hammer (1936). *Res. Bull. Ia agric. Exp. Sta.* No. 206.
(101) Sherman & Wing (1935). *J. Dairy Sci.* **18**, 657.
(102) —— —— (1937). *J. Dairy Sci.* **20**, 165.
(103) Davis & Tarr (1936). *Nature, Lond.*, **138**, 763.
(104) Sherman & Hodge (1936). *J. Dairy Sci.* **19**, 494. (Ab.)
(105) Jurukoff (1936). *Zbl. Bakt.* II, **95**, 324.
(106) Orla Jensen *et al.* (1936). *Zbl. Bakt.* II, **93**, 321.
(107) Eggerth (1935). *J. Bact.* **30**, 277.
(108) Pederson (1936). *J. Bact.* **31**, 217.
(109) Allen & Harrison (1936). *Ann. appl. Biol.* **23**, 546.
(110) Pederson (1937). *J. Bact.* **33**, 90.
(111) Wright (1934). *J. Path. Bact.* **39**, 359.
(112) Davis (1935). *J. Dairy Res.* **6**, 121.
(113) Minett *et al.* (1929). *J. comp. Path.* **42**, 213.
(114) Gibson & Muir (1935). *J. Hyg., Camb.*, **35**, 238.
(115) Minett (1936). *J. Hyg., Camb.*, **35**, 504.
(116) Stableforth (1936). *Proc. II. Int. Congr. Microbiol., London*, p. 139.
(117) Hansen (1935). *Tech. Bull. N.Y. agric. Exp. Sta.* No. 232.
(118) Cunningham (1936). *Ann. Conf. Soc. agric. Bact.* "The occurrence and detection of streptococcal mastitis."
(119) Hartmann (1936). *Milchw. Forsch.* **18**, 116.
(120) Pullinger (1935). *J. Dairy Res.* **6**, 369.

(121) Bryan (1934). *Vet. Med.* **29**, 524.
(122) Chaix (1935). *C.R. Acad. Sci., Paris*, **201**, 857 (*Chem. Abstr.* **30**, 1832, 1936).
(123) Fromageot & Piret (1936). *Arch. Mikrobiol.* **7**, 551.
(124) Tatum, Wood & Peterson (1936). *J. Bact.* **32**, 167.
(125) —— —— —— (1936). *Biochem. J.* **30**, 1898.
(126) Langlykke & Fred (1937). *J. Bact.* **33**, 102.
(127) Rosell (1933). *Action med.*, p. 56 (*Lait*, **15**, 660, 1935).
(128) Samtsevich (1934). *Mikrobiol.* **3**, 103 (*Exp. Sta. Rec.* **72**, 687, 1935).
(129) Elliker & Frazier (1937). *J. Bact.* **33**, 91.
(130) Whitehead & Cox (1935). *N.Z. J. Agric.* **50**, 107.
(131) Ritter & Christen (1935). *Landw. Jb. Schweiz.* **49**, 749.
(132) Harriman (1934). *Iowa St. Coll. J. Sci.* **9**, 155 (*Chem. Abstr.* **29**, 5193, 1935).
(133) Davis (1936). *Chemikerztg*, **60**, 778.
(134) Hansen (1936). *Hansen's Bull.* **21**, 8.
(135) Cox & Whitehead (1936). *N.Z. J. Agric.* **52**, 38.
(136) Whitehead & Cox (1935). *N.Z. J. Sci. Tech.* **16**, 319.
(137) Davis (1935). *Agric. Progr.* **12**, 138.
(138) Eagles, Okulitch & Campbell (1936). *Canad. J. Res.* B, **14**, 311.
(139) Hammer *et al.* (1935). *Res. Bull. Ia agric. Exp. Sta.* No. 191.
(140) Michaelian & Hammer (1936). *Res. Bull. Ia agric. Exp. Sta.* No. 205.
(141) Stahly *et al.* (1935). *J. Dairy Sci.* **18**, 473. (Ab.)
(142) van Beynum, J. (1933). *Versl. Ver. Proefzuiv. Hoorn*, 65 (*Lait*, **16**, 773, 1936).
(143) Vas & Csiszár (1936). *Milchw. Forsch.* **18**, 176.
(144) Brewer *et al.* (1937). *J. Bact.* **33**, 92.
(145) Hammer (1936). *J. Bact.* **31**, 479.
(146) —— (1935). *J. Dairy Sci.* **18**, 579.
(147) Davis (1936). *Dairy Ind.* **1**, No. 1, 9.
(148) Barnicoat (1937). *J. Dairy Res.* **8**, 61.
(149) Virtanen (1933). *Karjantuote*, **16**, 84.
(150) Powell (1936). *J. Dairy Sci.* **19**, 305.
(151) Pien (1934). *Bull. Ass. Chim. Sucr.* **51**, 497 (*Brit. chem. Abstr.* B, 77, 1935).
(152) (1936). *Nat. Butt. Cheese J.* **27**, No. 13, p. 12.
(153) (1936). *Nat. Butt. Cheese J.* **27**, No. 18, p. 26.
(154) Funder (1935). *C.R.* No. 27, *Dairy Exp. Sta., Norway* (*Lait*, **15**, 1106, 1935).
(155) Korolew, Wlasow & Babkina (1936). *Bull. Inst. Milchw. Vologda*, **81**, 121 (*Zbl. Bakt.* II, **95**, 434, 1937).
(156) Truninger (1935). *Landw. Jb. Schweiz*, **49**, 934 (*Chem. Abstr.* **30**, 3901, 1936).
(157) Heltstrand (1928). *Maelkeritidende*, **41**, 965.
(158) Mergner (1934). *Dtsch. Molkereiztg*, **55**, 1344.
(159) Zeiler *et al.* (1934). *Dtsch. Molkereiztg*, **55**, 446.
(160) Gorini (1936). *Milchw. Zbl.* **65**, 73.
(161) Hussmann (1935). *Milchw. Zbl.* **64**, 438.
(162) van Beynum & Pette (1934). *Versl. Landw. Onderzoek. Landb. Proefsta. Hoorn*, **40** C, 215 (*Zbl. Bakt.* II, **93**, 71, 1935).
(163) —— —— (1936). *Zbl. Bakt.* II, **94**, 413.
(164) Hostettler (1936). *Milchw. Zbl.* **65**, 258, 271.
(165) Frazier *et al.* (1935). *J. Dairy Sci.* **18**, 373.
(166) —— (1935). *J. Dairy Sci.* **18**, 503.
(167) Burkey, Sanders & Matheson (1935). *J. Dairy Sci.* **18**, 719.
(168) Frazier, Long & Johnson (1936). *J. Dairy Sci.* **19**, 535.
(169) Stölting (1935). Über die Streptokokken des normal reifenden Tilsiter Käses. Inaug. Diss. Kiel.
(170) Davis (1935). *J. Dairy Res.* **6**, 175.

(171) Bondioli (1931). *Ann. Ist. sper. Caseif. Lodi*, **9**, 31 (*Milchw. Forsch.* **17**, Ref. 354, 1936).
(172) Boysen (1936). *Milchw. Forsch.* **18**, 150.
(173) Hofmann (1935). Inaug. Diss. Kiel (*Zbl. Bakt.* II, **95**, 80, 1936).
(174) Kelly (1937). *J. Bact.* **33**, 90.
(175) Grimmer & Schmid (1936). *Milchw. Forsch.* **17**, 286.
(176) Sumner (1935). *Ann. Rev. Biochem.* **4**, 37.
(177) Parisi & de Vito (1934). *R. C. Accad. Lincei* (vi), **20**, Fasc. 1/2 (*Lait*, **16**, 882, 1936).
(178) Davis (1935). *J. Soc. chem. Ind., Lond.*, **54**, 631.
(179) Davies *et al.* (1937). *J. Dairy Res.* **8**, 92.
(180) Sherwood (1935). *J. Dairy Res.* **6**, 204.
(181) —— (1935). *J. Dairy Res.* **6**, 407.
(182) Hansen (1935). *Proc. West. Div. Amer. Dairy Sci. Ass.* **21**, 59 (*Chem. Abstr.* **30**, 3901, 1936).
(183) Lane & Hammer (1935). *Res. Bull. Ia agric. Exp. Sta.* No. 190.
(184) —— —— (1935). *Res. Bull. Ia agric. Exp. Sta.* No. 183.
(185) Sherwood (1936). *J. Dairy Res.* **7**, 271.
(186) Ramsbottom (1936). *J. Soc. chem. Ind., Lond.*, **55**, 717.
(187) Savini (1936). *Il "Gorgonzola" e gli altri formaggi erborinati.* Rome: Soc. anon. arte della Stampa, pp. 183.
(188) Golding (1934). *Iowa St. Coll. J. Sci.* **9**, 151 (*Chem. Abstr.* **29**, 5193, 1935).
(189) Demeter & Pfundt (1936). *Zbl. Bakt.* II, **95**, 54.
(190) Lane (1936). *J. Dairy Sci.* **19**, 498. (Ab.)
(191) Corminboeuf (1935). *Sci. Agric.* **15**, 329 (*Chem. Abstr.* **29**, 6316, 1935).
(192) Funder (1935). *Molkereiztg (Hildesh.)*, **49**, 78 (*Chem. Abstr.* **29**, 2612, 1935).
(193) Lane & Hammer (1936). *Iowa St. Coll. J. Sci.* **10**, 391.
(194) Hüttig (1934). *Molkereiztg*, **48**, 295.
(195) Platon (1934). *Svenska Mejeritidn.* **26**, 143 (*Lait*, **15**, 1016, 1935).
(196) van Beynum (1932). *Versl. Ver. Proefzuiv. Hoorn*, p. 199 (*Exp. Sta. Rec.* **72**, 686, 1935).
(197) Keilling (1934). *Ann. Falsif., Paris*, **27**, 600 (*Chem. Abstr.* **29**, 2612, 1935).
(198) Knudsen, Gormsen & Nielsen (1937). *K. VetHøjsk. Aarsskr.* p. 43.
(199) Phillips (1935). *J. Dairy Sci.* **18**, 471. (Ab.)
(200) Price (1936). *J. Dairy Sci.* **19**, 185.
(201) Corbett, Frazier & Price (1935). *J. Dairy Sci.* **18**, 472. (Ab.)
(202) Reid & Brock (1934). *Bull. Mo. agric. Exp. Sta.* No. 340, p. 43.
(203) Fabian & Severens (1935). *J. Dairy Sci.* **18**, 773.
(204) Koestler & Wegmüller (1934). *Landw. Jb. Schweiz*, p. 842.
(205) Spicer & Burgwald (1935). *Nat. Butt. Cheese J.* **26**, No. 21, pp. 14, 33.
(206) Hiscox (1936). *J. Soc. chem. Ind., Lond.*, **55**, 80. (Ab.)
(207) Davis (1936). *J. Soc. chem. Ind., Lond.*, **55**, 80. (Ab.)
(208) Davis & Mattick (1936). *Agric. Progr.* **13**, 126.
(209) Leitch (1936). *Scot. J. Agric.* **19**, 343.
(210) Dahlberg *et al.* (1936). *Tech. Bull. N.Y. agric. Exp. Sta.* No. 239.
(211) Sanders, Matheson & Burkey (1936). *J. Dairy Sci.* **19**, 395.
(212) McDowall, Dolby & McDowell (1937). *J. Dairy Res.* **8**, 31.
(213) Sommer & Matsen (1935). *J. Dairy Sci.* **18**, 741.
(214) Anderson *et al.* (1936). *Bull. Storrs agric. Exp. Sta.* No. 211.
(215) Deger (1933). *Süddtsch. Molkereiztg*, **54**, Spec. No. 105 (*Lait*, **16**, 431, 1936).
(216) Caserio (1936). *Z. Untersuch. Lebensmitt.* **71**, 254.
(217) Demeter (1937). *Molkereiztg (Hildesh.)*, Nos. 4, 5, and 6.
(218) Fay (1935). *J. Dairy Sci.* **18**, 603.
(219) Moir & Russell (1937). *N.Z. J. Agric.* **54**, 144.

(220) Rice (1937). Dairy Research Laboratory, Department of Agriculture, Brisbane, Queensland. Private communication.
(221) Stark & Scheib (1936). *J. Dairy Sci.* **19**, 191.
(222) Wood & Thornton (1935). *Canad. J. Res.* **12**, 286.
(223) Thornton & Wood (1935). *Canad. J. Res.* **12**, 295.
(224) Riddet & Neill (1936). *N.Z. J. Agric.* **53**, 129.
(225) Neill (1935). *N.Z. J. Agric.* 51, 22.
(226) Herreid, Macy & Combs (1934). *Tech. Bull. Minn. agric. Exp. Sta.* No. 97.
(227) Virtanen & Tarnanen (1936). *Acta chem. fenn.* B, **9**, No. 2.
(228) Slatter (1936). *Nat. Butt. Cheese J.* **27**, No. 20, p. 20; No. 21, p. 18.
(229) Michaelian & Hammer (1935). *Res. Bull. Ia agric. Exp. Sta.* No. 179.
(230) Barnicoat (1935). *J. Dairy Res.* **6**, 397.
(231) Randell (1936). *J. Aust. Inst. agric. Sci.* **2**, 23 (*Brit. chem. Abstr.* B, 567, 1936).
(232) Rice (1937). Dairy Research Laboratory, Department of Agriculture, Brisbane, Queensland. Private communication.
(233) Bouska (1935). *Food Industr.* **7**, 381. (*Chem. Abstr.* **29**, 8161).
(234) Hiscox (1936). *J. Dairy Res.* **7**, 238.
(235) Platon & Olsson (1936). *Medd. Cent. Anst. Försöksv. Jordbr., Stockh.*, p. 466 (*Lait*, **16**, 778, 1936).
(236) Vernon (1935). *J. Dairy Res.* **6**, 154, 168.
(237) —— (1936). *Dairy Ind.* **1**, 21, 56, 125.
—— (1937). *Dairy Ind.* **2**, 63.
(238) Cruess-Callaghan (1935). *Sci. Proc. R. Dublin Soc.* **21** (N.S.), 253.
(239) Storen & Nestaas (1935). *Medd. Norg. LandbrHøisk. Mejeriavdel.* No. 6. (*Chem. Abstr.* **29**, 8160.)
(240) Sorkin (1935). *Mendel Bull.* **7**, 69 (*Chem. Abstr.* **29**, 5192, 1935).
(241) Overman (1936). *Nat. Butt. Cheese J.* **27**, No. 24, p. 6.
(242) Guthrie, Scheib & Stark (1936). *J. Dairy Sci.* **19**, 267.
(243) Gilmour & Arup (1935). *Ice cold Stor.* **38**, 120 (*Chem. Abstr.* **29**, 6964, 1935).
(244) Kellermann (1936). *Molkereiztg*, **50**, 526 (*Lait*, **16**, 1108, 1936).
(245) Ause & Macy (1934). *Amer. Cr. and Poult. Prod. Rev.* **79**, 190 (*Exp. Sta. Rec.* **75**, 688, 1936).

IV. Pasteurization and other processes

(1) Hiscock (1935). *Rep. int. Ass. Dairy Insp., Wash.*, **24**, 46.
(2) Bryan, Robinson & Severens (1936). *Quart. Bull. Mich. agric. Exp. Sta.* **19**, 44.
(3) Caserio (1934). *Ann. Igiene* (*sper.*), **44**, 1013.
(4) Mattick (1937). National Institute for Research in Dairying (6 pp.).
(5) Mattick & Hiscox (1937). *World's Dairy Congr. Sci. Rep.* **3**, 344.
(6) Meyn (1935). *Dtsch. tierärztl. Wschr.* **43**, 737 (*Z. Fleisch- u. Milchhyg.* **46**, 159, 1936).
(7) Bertucci (1934). Thèse Doc. Vét. Toulouse (*Lait*, **16**, 34, 1936).
(8) Lembke & Claussen (1937). *Z. Fleisch- u. Milchhyg.* **47**, 237.
(9) Yale (1935). *Lait*, **15**, 466, 612, 710.
(10) Anonymous (1936). *Milk Pl. Mon.* **25**, No. 3, p. 48.
(11) Putnam (1935). *Proc. int. Ass. Milk Dlr*, 28th Ann. Conv. Plant Sect. p. 138.
(12) Belotti (1933). *Profilassi*, **6**, 308 (*Biol. Abstr.* **10**, 691, 1936).
(13) Wedemann (1935). *Reichsgesundheitsblatt*, **10**, 859 (*Z. Untersuch. Lebensmitt.* **71**, 208, 1936).
(14) Farrall (1936). *Milk Pl. Mon.* **25**, No. 9, p. 28.
(15) Lambert (1935). *Milk Ind.* **16**, No. 5, p. 85.
(16) Lythgoe (1934). *Rep. int. Ass. Dairy Insp., Wash.*, **23**, 81.
(17) Savage (1936). *Publ. Hlth*, **49**, 260.

(18) Frank (1936). *Rep. int. Ass. Dairy Insp., Wash.* (*Milk Pl. Mon.*, **26**, 48, 1937).
(19) Ministry of Health (1935). Circular 1473 (*Analyst*, **60**, 763, 1935).
(20) Scott & Wright (1935). *Bull. Hannah Dairy Inst.* No. 6.
(21) Kay & Graham (1935). *J. Dairy Res.* **6**, 191.
(22) Helmreich (1936). *Z. Fleisch- u. Milchhyg.* **46**, 154.
(23) Eble & Pfeiffer (1936). *Z. Fleisch- u. Milchhyg.* **46**, 340.
(24) Slanetz (1936). *J. Bact.* **32**, 127.
(25) Breed *et al.* (1935–6). *Yearb. Amer. publ. Hlth Ass.* **6**, 103.
(26) Sherman (1935). *Proc. int. Ass. Milk Dlr*, **28**, Lab. sect., p. 51.
(27) Minkin & Burgwald (1935). *J. Dairy Sci.* **18**, 474.
(28) Demeter & Eisenreich (1936). *Dtsch. Molkereiztg*, **57**, 1533.
(29) Vernon & Walker (1936). *Ann. Conf. Soc. Agric. Bact.* "*Coli* contamination in holder pasteurized milk."
(30) Chilson & Yale (1935). Soc. Amer. Bact., Central N.Y. State Branch (*J. Bact.* **31**, 103, 1936).
(31) Chilson, Yale & Eglinton (1936). *J. Dairy Sci.* **19**, 337.
(32) Graham (1936). *Canad. publ. Hlth J.* **27**, 81.
(33) Alexander & Milone (1936). Soc. Amer. Bact., Eastern N.Y. Branch (*J. Bact.* **31**, 574, 1936).
(34) Forgacs (1935). *Z. Untersuch. Lebensmitt.* **69**, 248.
(35) Haines (1935). *Rep. Food Invest. Bd, Lond.*, p. 31 (*Brit. chem. Abstr.* A, **3**, 72, 1937).
(36) Anonymous (1936). *Milk Pl. Mon.* **25**, No. 12, p. 68.
(37) Beckwith & Weaver (1936). *J. Bact.* **32**, 361.
(38) Anonymous (1937). *Milk Tr. Gaz.* **10**, No. 171, p. 4.
(39) Haines (1935). *Rep. Food Invest. Bd, Lond.*, p. 30 (*Brit. Chem. Abstr.* A, **3**, 72, 1937).
(40) Heine (1935). *Z. Fleisch- u. Milchhyg*, **45**, 381.
(41) van Raalte (1936). *Food Manuf.* **11**, 359.
(42) Mattick (1936). *J. Minist. Agric.* **43**, 516.
(43) Müller (1936). *Z. Fleisch- u. Milchhyg*, **46**, 276.
(44) Anonymous (1936). *Dtsch. Bergwerks Ztg* (*Milk Ind.* **17**, No. 5, p. 45, 1936).
(45) Anonymous (1937). *Milk Dlr*, **26**, No. 6, p. 108.
(46) Harding (1937). *Bull. Dairy Res. Bur., Mathews Co.* (*Detroit*), **16**, 24.
(47) da Silva-Mello (1936). *Med. Klinik.* **32**, 586 (*Zbl. Bakt.* II, **95**, 268, 1936).
(48) Nichols (1936). *J. Soc. chem. Ind., Lond.*, **55**, 78.
(49) Olson & Hammer (1935). *J. Dairy Sci.* **18**, 474.
(50) Damm & Hornbostel (1936). *Milchw. Forsch.* **18**, 107.
(51) McMaster (1932–3). *Rep. Fruit Veg. Pres. Sta., Campden*, p. 71.
(52) Brennert (1935). *Tech. Publ. Int. Tin. Res. Coun.* Series D, **2**.
(53) Jackson, Howat & Hoar (1936). *J. Dairy Res.* **7**, 284.
(54) Thomas (1935). *Med. Offr.* **54**, 132 (*Bull. Hyg.* **11**, 141, 1936).
(55) Anderson (1935). *J. R. sanit. Inst.* **55**, 592.
(56) Fabian (1935). *Proc. Ann. Conv. int. Ass. Ice Cr. Manuf.* **35**, 56 (*J. Dairy Sci.* **19**, Abstr. **126**, 1936).
(57) Sommer (1936). *Ice Cr. Field*, **28**, No. 6, p. 9 (*J. Dairy Sci.* **19**, Abstr. 229, 1936).
(58) Fisher (1935). *Proc. Ann. Conv. int. Ass. Ice Cr. Manuf.* **35**, 60 (*J. Dairy Sci.* **19**, Abstr. 129, 1936).
(59) Grimes (1936). *Ann. Conf. Soc. Agric. Bact.* "A comparison of lactose nutrient agar and tryptone glucose agar."
(60) Babel (1936). *Ice Cr. Tr. J.* **32**, No. 9, p. 35 (*J. Dairy Sci.* **19**, Abstr. 265, 1936).
(61) Fay (1935). *Ice Cr. Rev.* **18**, No. 7, p. 38 (*Exp. Sta. Rec.* **74**, 845, 1936).
(62) Martin (1936). *Ice Cr. Tr. J.* **32**, No. 5, p. 19 (*J. Dairy Sci.* **20**, Abstr. 30, 1937).

(*MS. received for publication* 27 *September* 1937)

CAMBRIDGE: PRINTED BY
W. LEWIS, M.A.
AT THE UNIVERSITY PRESS